Development of wound healing spray from keratin protein

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ARTICLE INFO

Article history:
Received 25 March 2020
Received in revised form
10 April 2020
Accepted 30 May 2020

Keywords:
Wound healing spray
Keratin protein
Chicken feather
Manuka honey
Garcinia Mangostana L.
Capryl glycol
Ethylhexylglycerin
Propylene glycol

ABSTRACT

Wound healing spray was developed from keratin protein extracted from a chicken feather in the study. Keratin is the most abundant protein in epithelial cells. Keratin was proven for wound healing ability because it can activate the keratinocyte in the skin responsible for wound healing. However, keratin wound healing spray is new to the market and has the potential to heal the wound gentle and pain-free. This study synthesized the keratin wound healing spray from chicken feathers and determined the character of the keratin wound healing spray. Keratin protein was extracted from chicken feathers. The extracted keratin solution was concentrated to the desired concentration by a rotary evaporator. The wound healing spray was synthesized by mixing the desired antimicrobial agent with keratin solution like manuka honey, *Garcinia Mangostana* L., capryl glycol, ethylhexylglycerin and propylene glycol at a concentration of 13.0 w/w%, 0.5 w/w%, 1.0 w/w%, 2.0 w/w% and 2.0 w/w%, respectively, and the characteristic was determined. The result has shown that the keratin protein is maintained in the wound healing spray after mixing with an antimicrobial agent according to the formulation from the FTIR result. The wound healing spray does not contain heavy metals like cadmium and lead. Still, copper, iron and zinc were present within the maximum daily level of vitamins and minerals for adults allowed in health supplements by the National Pharmaceutical Regulatory Division of Malaysia. The pH of the keratin wound healing spray was maintained at a pH of around 5.5. The density and the viscosity of the keratin wound healing spray were higher than the deionized water. In conclusion, the Keratin wound healing spray was synthesized, and it is safe for the consumer. The wound healing ability of the keratin wound healing spray needs to carry out the in vivo clinical test for future study.

1. Introduction

Keratin is a group of filament-forming proteins with high sulphur content and insoluble. It was usually constituting in the bulk of epidermal such as claw, turtle shell and feather. It is the most abundant protein in epithelial cells because it's the largest subgroup of intermediate filament proteins that protect epithelial cells from nonmechanical and mechanical stress, which is the primary function of keratins (Coulombe & Omary, 2002; Husain et al., 2018; Abayomi et al., 2016). Keratin is classified as α-Keratin and β-Keratin. α-Keratin including wools, quills, hair, fingernails, horns and hooves, while β-Keratin, including feathers, avian beaks.

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and claws, reptilian claws and scales (Bin et al., 2016; Alashwal et al., 2019).

Keratin has been identified to have the wound-healing ability. Keratin can activate the keratinocyte in wound healing. Keratin extracted from ovine wool can treat an acute wound, chronic wound and some skin disorders, epidermolysis bullosa (Kelly, 2016; Alashwal et al., 2020). Patients reported a faster healing rate and more resilient healed skin for Epidermolysis bullosa, a genetically inherited condition that will cause skin fragility and minor trauma leading to skin loss, damage, and woundng (Enter et al., 2015; Yu et al., 2017).

The Keratin can be extracted from the chicken feather because chicken feathers contain a high level of protein so that it becomes the suitable raw material for the extraction of Keratin (Arun et al., 2012). It is shown that Malaysia poultry consumption is 36 kg per capita per year which is significantly higher than Indonesia. 9 kg per capita per year. Hence, colossal poultry consumption in Malaysia can maintain chicken feathers' sustainable raw material supply in extracting raw Keratin (Wahyono & Utami, 2018). The chicken feather that used in extracting the keratins protein is biodegradable. Moreover, the optimization of resources is one of the challenges in the world. By using chicken feather in the extraction of keratin protein and converting into a functional product, the resources can be utilized.

Besides the wound healing ability of the keratin protein that can activate keratinocytes, pathogenic bacteria are the leading cause of wound and unhealed wounds. The most common pathogens are Enterococci, Escherichia, Pseudomonas, Klebsiella, Enterobacter, Proteus and Acinetobacter (Farrag et al., 2016). Moreover, several studies investigate the microbiome of different chronic wounds, including diabetic foot ulcers and pressure ulcers. The main bacteria found is Staphylococcus, Streptococcus, Corynebacterium, Pseudomonas, and various anaerobes (Xu & Hsia, 2018). The most common bacteria found in diabetic foot infections are Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, beta-hemolytic streptococci, Proteus species, Methicillin-resistant S. aureus, Enterococcus, etc. (Banu et al., 2015). Hence, the wound healing spray needs to have wound healing ability by keratin protein and the antimicrobial agent that can inhibit the growth of microbial that can cause the wound infection that makes the wound unhealed and infected.

Caprylyl glycol belongs to the short-chain 1,2-glycols group are usually apply as hair or skin conditioning agents and viscosity agents in the cosmetic product, but at the same time, it also acts as the cosmetic preservatives. The cosmetic Ingredient Review (CIR) of the United States of America reported dermally absorbed and negative oral toxicity data on shorter chain 1,2- glycol, caprylyl glycol. Hence, caprylyl glycol is safe for usage in cosmetic products. The minimum inhibition concentration (MIC) of caprylyl glycol to inhibit Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans within 1 day Aspergillus niger within 28 days was a concentration of 0.5%. Next, ethylhexyglycerin inhibited the same microorganisms in the same period at the minimum concentration of 1.5% (Lawan et al., 2009). Besides, the minimum inhibition concentration of Garcinia Mangostana L. toward gram-positive bacteria (L. monocytogenes and S. Aureus) and gram-negative bacteria (E. coli and Salmonella sp.) should be more than 3.13 mg/ml or 0.00313 mg/L (Palakawang et al., 2010).

Propylene glycol (PG) is aliphatic alcohol produced from propylene oxide and water reactions. It acts as a skin-conditioning agent, viscosity decreasing agent, solvent and fragrance ingredient in cosmetics. The Cosmetic Ingredient Review (CIR) had concluded that PG is nontoxic and noncarcinogenic. Hence, it is safe for application in cosmetic formulations with non-irritating at a concentration of 0.0008 to 99%. There is no evidence of sensitization of 86% PG tested using a stick antiperspirant. It also acts as a penetration enhancer for some chemicals under conditions that can enhance the penetration of ingredients into the epidermis but has not been definitively identified. It was reported that 57% dermal penetration of PG from a ternary cosolvent solution through hairless mouse skin over 24 hours without PG reaching the dermis in human skin (Fiume et al., 2012).

Garcinia Mangostana L. (Mangosteen) has wound healing ability and can inhibit Staphylococcus aureus and Escherichia coli with a minimum inhibit concentration of 0.313% (Palakawang et al., 2010). A study reported the wound healing promotion activity of Garcinia Mangostana L. in Sprague-Dawley rates by inducing skin epithelization and wound contraction. The xanthones isolated from Garcinia Mangostana L. have antimicrobial and anti-inflammatory activities. It was suggested that the Garcinia Mangostana L. could treat the open wound due to its efficacy in increasing fibroblast cell migration (Wisujitrat & Waranuch, 2019).

Table 1 List of microorganisms sensitive to Manuka kinds of honey (Sarfraz & Nor Hayati, 2013).

<table>
<thead>
<tr>
<th>Gram-Positive Strains</th>
<th>Gram-Negative Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pyogenes</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>Acinetobacter baumannii</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>Salmonella enterica Serovar</td>
</tr>
<tr>
<td>Methicillin-resistant</td>
<td>Typhi</td>
</tr>
<tr>
<td>Staphylococcus aureus (MRSA)</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>Staphylococcus aureus (CONS)</td>
<td>Shigella flexneri</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Staphylococcus mutans</td>
<td>Enterococcus</td>
</tr>
<tr>
<td>Streptococcus sobrinus</td>
<td>Enterobacter cloacae</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>Shigella sonnei</td>
</tr>
<tr>
<td>-</td>
<td>Salmonella typhi</td>
</tr>
<tr>
<td>-</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>-</td>
<td>Burkholderia cepacia</td>
</tr>
<tr>
<td>-</td>
<td>Helicobacter pylori</td>
</tr>
<tr>
<td>-</td>
<td>Campylobacter spp.</td>
</tr>
<tr>
<td>-</td>
<td>Porphyromonas gingivalis</td>
</tr>
</tbody>
</table>

Furthermore, manuka honey is mono-floral honey produced from the nectar of the manuka tree. According to some research
about the microbial ability of manuka honey, it is effective against some human pathogens, including Escherichia coli, Enterobacter aerogenes, Salmonella typhimurium, methicillin-resistant S. aureus, and β-haemolytic streptococci, and vancomycin-resistant Enterococci (VRE). Minimum Inhibitory Concentration (MIC) observed for manuka honey is 12.5 v/v % (Mandal & Mandal, 2011). Table 1 showed the list of the microorganism that are sensitive to manuka honey at 12.5 v/v%. There is much research that has shown the ability of wound healing from keratin protein. However, the pharmaceutical product developed from Keratin the market is new in the market. Examples of products from Keratin developed by Molecular Biologics are Keragel, KeragelT and Keramatrix. These products showed positive wound healing ability as claimed by the company and clinical test. However, currently, there is no wound healing spray using keratin protein available on the market. Wound healing spray causes less pain to the patient because it doesn’t need direct contact like gel or ointment, especially for chronic wounds. If the keratin wound healing spray is developed with optimum healing ability, it benefits the patient, especially chronic wounds.

Chronic wounds are the wounds that fail in typical wound healing phases in an orderly and timely manner. Application of wound healing products is needed to accelerate the wound healing of chronic wounds. Keratin is the original component in epithelial cells, and many researchers have shown its ability to accelerate wound healing by activating the keratinocyte. The optimum concentration of the Keratin used in a spray has to be determined. Although Keratin has shown no adverse effect on the human skin, the different compositions have different wound healing ability. Different compositions and components like antimicrobial agents needed to be discussed to find the most suitable component and composition that can increase the performance of wound healing spray from Keratin. They do not cause harm to the user. The study focus on the synthesis and characterization of the wound healing spray developed using keratin protein from chicken feathers such as Fourier-Transform Infrared Spectroscopy (FTIR), pH, the mass of keratin protein in the wound healing spray sample and heavy metal test.

2. Materials and Methods

2.1. Materials

Sodium hydroxide (pellet; purity, 99% NaOH), hydrochloric acid (purity, 36.5 – 38%) and propylene glycol (purity, >99.5%) were purchased from Sigma-Aldrich Co, Malaysia. Caprylyl glycol (purity, 99%) and ethylhexylglycerin (purity, 99%) were purchased from Gardner Glybol Enterprise, Kuantan, Pahang, Malaysia. Manuka Honey (MG250+) was purchased from Manuka Health Co. New Zealand. Garcinia Mangostana L. (powder) was obtained from Furlot Bio extracts Sdn. Bhd. The chicken feather for the extraction of Keratin was procured from Balok Poultry Farm Sdn. Bhd. Kuantan, Malaysia.

2.2. Keratin protein preparation from chicken feather

A palm mill near Gambang, Pahang, Malaysia provided The keratin protein extraction was prepared inside the Faculty of Chemical & Process Engineering Technology laboratory at Universiti Malaysia Pahang. 50g of cleaned, dried and blended chicken feather was added into 1L of 1N sodium hydroxide solution in a conical flask. The solution was incubated at 60°C by magnetic stirrer with heating and ceramic heating plate (C-MAG HS7 by IKA) and continuous stirring at 600rpm by overhead stirrer (RW20 Digital by IKA) for six hours. After that, the solution was filtered by stainless steel filter and centrifuged at 10,000rpm (Centrifuge 5810 R by Eppendorf) for 10 minutes. The solution was collected after centrifugation. The keratin protein mass concentration in the keratin solution was determined by drying 5g of extracted keratin solution in universal oven (Heraeus) under a temperature of 104°C for 24 hours. The mass concentration of keratin protein in the solution was calculated using Eq. 1.

\[ C_{Keratin} = \frac{M_{\text{Dried}}}{M_{\text{Initial}}} \times 100\% \]  

Where:

- \( C_{Keratin} \) = Concentration of keratin, w/w%
- \( M_{\text{Dried}} \) = Dried mass of Keratin, g
- \( M_{\text{Initial}} \) = Initial Mass of keratin solution, g

2.3. Wound healing spray production

The keratin protein was evaporated by using a rotary evaporator (Rotavapor R-100 by Buchi) at 59°C to obtain the concentrated keratin solution as the bottom product.

<table>
<thead>
<tr>
<th>Formulation (w/w%)</th>
<th>Keratin + Manuka Honey (H1)</th>
<th>Keratin + Garcinia Mangostana L. (KM1)</th>
<th>Keratin + Chemicals (C1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin Solution</td>
<td>87.0</td>
<td>99.5</td>
<td>95.0</td>
</tr>
<tr>
<td>Manuka Honey</td>
<td>13.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Garcinia Mangostana L.</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Caprylyl Glycol</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethylhexylglycerine</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The mass evaporated distillate (sodium hydroxide) needed to be evaporated from the keratin solution to achieve the desired concentration was calculated using Eq. 2.

\[ M_{\text{Distillate}} = M_{\text{Initial}} - \left( M_{\text{Initial}} \times \frac{C_{\text{Initial}}}{C_{\text{Desired}}} \right) \]  

Where:

- \( M_{\text{Distillate}} \) = Mass of Distillate, g
- \( M_{\text{Initial}} \) = Initial Mass of keratin solution, g
- \( C_{\text{Initial}} \) = Initial keratin concentration, w/w%
- \( C_{\text{Desired}} \) = Desired keratin concentration, w/w%

Caprylyl glycol, ethylhexylglycerin, propylene glycol, manuka honey and Garcinia Mangostana L. were measured and mixed into
the keratin solution and stirred by a magnetic stirrer (C-MAG HS7 by IKA) for 30 minutes under room temperature (30°C) according to the formulation.

2.4 Mass Concentration of keratin protein

The mass of keratin protein in each of the wound healing sprays was calculated using Eq. 3.

\[ C_{\text{Keratin Protein}} = \frac{M_{\text{Keratin Solution}} \times C_{\text{Initial Keratin}}}{M_{\text{Total}}} \]  

(3)

Where:
- \( k_{\text{eratin protein}} \) = mass of keratin protein in the wound healing spray sample, g
- \( M_{\text{Total}} \) = Total mass of wound healing spray sample, g
- \( C_{\text{Keratin Protein}} \) = Mass concentration of keratin solution in the wound healing spray, w/w%
- \( C_{\text{Initial Keratin}} \) = Initial keratin concentration, w/w%

2.5 Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The Fourier-Transform Infrared Spectroscopy (FTIR) analysis examined the chemical functional group available in the keratin wound healing spray sample. The FTIR graph from the analysis was used to ensure the keratin protein still available in the wound healing spray sample after the mixing according to the formulation. The FTIR spectra were obtained in the range of wavelength 4000 to 400 cm\(^{-1}\) and it was carried out by Lab Technician at the Centre of Excellence for Advanced Research in Fluid Flow (CARIFF) at Universiti Malaysia Pahang.

2.6 Heavy metal test

The heavy metal test was to test the heavy metal such as cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) available in the wound healing spray sample that potential to be toxic to the consumer of the wound healing spray. The heavy metal may be contained in the chemical used during the production of wound healing spray. Inductively Coupled Plasma analyzed the heavy metal concentration – Optical Emission Spectrometry (ICP-OES) by Lab Technician in Centre of Excellence for Advanced Research in Fluid Flow (CARIFF) at Universiti Malaysia Pahang.

2.7 Wound Healing Spray Characterization

The wound healing spray was to analyze the characteristic of the wound healing spray such as pH, viscosity and density. The pH test was carried out to determine the pH of the wound healing spray. The pH of wound healing spray was analyzed by using a pH meter (Mettler Toledo). Besides, the viscosity of wound healing spray was analyzed by Rheometer (DV-111 Ultra Programmable Rheometer by Brookfield) at 200rpm using spindle number 6 at 27°C. Next, the density was determined by measuring the final mass using a weighing balance (DR-200 by AND) and volume of the wound healing spray produced using a measuring cylinder.

3. Results and Discussion

3.1 Physical observation of keratin wound healing spray

Table 2 showed the wound healing spray synthesized. The wound healing spray at the left was sample C1 (Keratin + Caprylyl Glycol + Ethylhexylglycerin + Propylene Glycol), middle was sample KM1 (Keratin + Garcinia Mangostana L.), and right was sample H1 (Keratin + manual honey). Sample C1 showed orange colour, and there was one layer of solution at the top. Hence, the antimicrobial agent and the keratin solution didn’t mix homogeneously. Next, the sample KM1 showed dark brown colour due to the dark brown colour of Garcinia Mangostana L. powder, and there were two layers found when the solution was left stationary for few hours. Moreover, sample H1 showed brown colour and the honey mixed homogeneously with the keratin solution. Hence, the keratin wound healing spray was suggested to be shaken before applying it to the wound.

![Fig. 1 Keratin wound healing spray sample.](image)

3.2 Mass concentration of keratin protein

Mass concentration of keratin solute in wound healing spray was calculated using Eq. 3. The mass concentration of keratin protein solute in the keratin protein solution is 8.7593 ±0.1231 w/w%. Table 3 showed the mass concentration of keratin protein solute in each wound healing spray sample. The wound healing spray samples were synthesised using the exact source of concentrated keratin protein solution. The different mass concentration of the keratin solute in each sample was due to the different mass of keratin solution used in each sample according to the formulation.
Table 3 Mass concentration of keratin protein solute in wound healing spray.

<table>
<thead>
<tr>
<th>Sample</th>
<th>H1</th>
<th>KM1</th>
<th>C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of Keratin Solution Used (g)</td>
<td>87.83</td>
<td>99.61</td>
<td>95.69</td>
</tr>
<tr>
<td>Total Mass of Spray (g)</td>
<td>105.61</td>
<td>104.83</td>
<td>108.25</td>
</tr>
<tr>
<td>Mass Concentration of Keratin Solute in Spray (w/w%)</td>
<td>7.2846</td>
<td>8.3231</td>
<td>7.7430</td>
</tr>
</tbody>
</table>

3.3. Fourier-transform infrared spectroscopy (FTIR) analysis

Figures 2, 3, and 4 showed the FTIR analysis result for the wound healing spray samples H1, KM1 and C1. FTIR analysis results for wound healing spray samples H1, KM1 and C1 have peaks at wavelength 3331.61 cm\(^{-1}\), 3346.74 cm\(^{-1}\), and 3346.30 cm\(^{-1}\), respectively. These peaks were corresponding to the secondary Amide. The peaks located in the 3310—3350 cm\(^{-1}\) correspond to the secondary Amide with the N-H bond.

Furthermore, the wound healing spray samples H1, KM1 and C1 have another peak at 1636.25 cm\(^{-1}\), 1635.90 cm\(^{-1}\), and 1636.25 cm\(^{-1}\), respectively. These peaks were corresponding to the Amide I band. The Amide I band’s keratin structure originates from the C=O stretching vibration, and its frequency is because of the hydrogen-bonding pattern and backbone conformation (Aluigi et al., 2007). The Amide I band (N-H bond). It is located at 1600 – 1700 cm\(^{-1}\) and is mainly connected with C-O stretching vibration (Wang & Cao, 2012). The Amide I peak corresponding to \(\alpha\)-helix structure protein is 1650-1656 cm\(^{-1}\) and \(\beta\)-pleated sheet structure protein is 1610 – 1640 cm\(^{-1}\). The FTIR result obtained from three samples showed peaks at 1636.25 cm\(^{-1}\) and 1635.90 cm\(^{-1}\). Hence, it is confirmed that the Amide I band represents the \(\beta\)-pleated Sheet keratin protein structure in the sample (Wan et al., 2008).

Besides, honey comprising carbohydrate structure at the peak at 1061 cm\(^{-1}\) analyzed from the keratin wound healing spray sample H1 showed that the sample contain C-O stretch in the C-OH group and the C-C stretch in carbohydrate structure (Anjos et al., 2015).

Fig. 2 FTIR analysis result for keratin wound healing spray sample H1 (Keratin + Honey).

3.4. Heavy metal test

The correlation coefficient of the calibration curve obtained from the standard solution preparation for the ICP-OES test for cadmium, copper, iron, lead, and zinc were 0.997178, 0.998688, 0.998914, 0.998826, and 0.998529, respectively. Hence, it is shown that the result of ICP-OES was accurate. Table 4 showed that all three-keratin wound healing spray samples do not have cadmium and lead. The maximum cadmium and lead consumed by humans daily is suggested to be 0.0013 to 0.56 μg/day and 0.0034 to 11.88 μg/day, respectively (Nessa et al., 2016).

Besides, all three-keratin wound healing spray samples contain 2.083 – 5.484 mg/L of iron and 1.147-1.584 mg/L of zinc, while sample C1 contain 0.343 mg/L of copper. The maximum daily level of vitamins and minerals for adults allowed in health supplements suggested by the National Pharmaceutical Regulatory Division of Malaysia for copper, iron and zinc are 2 mg/day, 20mg/day, and 15mg/day, respectively. The volume of each spray is expected to be lower than 1 mL. Hence, the copper, iron and zinc concentrations in the keratin wound healing spray sample were low compared to the standard.

Table 4 Heavy metal test result from ICP-OES analysis.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
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</table>
3.5. Wound healing spray characterization

Slightly acidic at pH 5.5 reported decreased bacterial growth rate for E. coli, S. aureus and may inhibit P. aeruginosa. It is also beneficial to keratinocytes’ in vitro growth and helps in wound healing (Varghese et al., 1986). pH is highly related to wound healing because a lower pH value can inhibit human pathogenic bacteria because it needs pH values above 6 to grow on the wound (Table 5). High bacterial load characterized by pH above 7.3 for infected wounds and chronic wounds (Schneider et al., 2006). Hence, three wound healing spray samples were neutralized to pH around 5.5 by hydrochloric acid.

Besides, the density of water at 30°C is 0.99568 g/cm³ (Geankoplis, 2014). The density for all three-keratin wound healing spray samples was higher than the density of water. The Keratin wound healing spray sample KM1 and C1 were almost the same, while the density of sample H1 was slightly higher than in another sample. This is because the density of the manuka honey was higher, and the concentration of keratin solution in sample H1 was lower compared to another sample.

The viscosity was analyzed by using spindle number 6 and a rotation speed of 200rpm at 27°C. The torque was maintained at more than 10% at a rotation speed of 200rpm when the viscosity of all three-keratin wound healing spray samples was recorded to obtain an accurate reading. The viscosity of deionized water at 27°C at 200rpm was 1.74cP from the analysis. Hence, all three-keratin wound healing spray samples have higher viscosity as compared to deionized water. The keratin wound healing spray sample C1 has the lowest viscosity among the three keratin wound healing spray samples. The viscous solution be thick and sticky so that it has a longer contact time with the wound when sprayed.

Table 5 Wound healing spray characterization.

<table>
<thead>
<tr>
<th>Keratin + Honey (H1)</th>
<th>Keratin + Garcinia Mangostana L. (KM1)</th>
<th>Keratin + Chemicals (C1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Density (g/cm³)</td>
<td>Viscosity (cP)</td>
</tr>
<tr>
<td>5.72</td>
<td>1.0776</td>
<td>2.31</td>
</tr>
<tr>
<td>5.29</td>
<td>1.0379</td>
<td>2.23</td>
</tr>
<tr>
<td>5.34</td>
<td>1.0309</td>
<td>2.07</td>
</tr>
</tbody>
</table>

4. Conclusion

In conclusion, the keratin wound healing spray was suggested to be shaken before applying it to the wound. The keratin wound healing spray developed from the study contain the keratin protein after mixing with antimicrobial agents such as manuka honey, Garcinia Mangostana L., caprylyl glycol, ethylhexyglycerine, and propylene glycol, according to the FTIR result. The wound healing spray does not contain heavy metals like cadmium and lead that are toxic and unsuitable for the pharmaceutical product. The other heavy metal such as copper, iron and zinc in the wound healing spray was significantly lower than the maximum daily levels of minerals for adults in health supplements allowed by National Pharmaceutical Regulatory Division of Malaysia. In-vitro or antimicrobial susceptibility test should be conducted to determine the antimicrobial activity of each wound healing spray toward the microbial that cause the chronic wound to be infected and unhealed. Next, the in-vivo test should be conducted to determine the wound healing ability of each Keratin wound healing spray developed from keratin protein according to the formulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgements

The author thanks the Faculty of Chemical & Process Engineering Technology for providing the facility for laboratory work and the financial assistant through research funding. Besides, K.W. Chin would like to thank Mohamed Saad Bala and Basma Y. Alashwal for their help throughout the study.

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Maejo International Journal of Energy and Environmental Communication


