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Synthesis and Characterization of *Aloe vera* – Chitosan – Snail Mucus as *Staphylococcus aureus* Anti-bacterial Topical Treatment

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ABSTRACT
An open wound not treated immediately and properly can cause an infection. It is primarily caused by the bacteria activity that grows in the wound, such as the growth of *Staphylococcus aureus*. This study aims to synthesize and characterize aloe vera gel, chitosan and snail mucus as anti-staphylococcal aureus. In this study, the synthesis of aloe vera gel, chitosan and snail mucus were carried out with variations concentrations of snail mucus. The resulting gel preparations were characterized physically and chemically. It was shown that the functional group of the gel preparation experienced a wave number shift, indicating a chemical reaction between aloe vera, chitosan and snail mucus. The antibacterial test showed that the diameter of the highest inhibition on the growth of *Staphylococcus aureus* was in a ratio of 1:1:1 *in vitro*.

Keywords: aloe vera-chitosan-snail mucus; Staphylococcus aureus; anti-bacteria

1. INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that becomes a leading cause of 20 - 50/100,000 bacteraemia cases which 10% - 30% of patients succumb to the infection annually [1-2]. Its infection can be superficial and invasive due to its ability to adapt its metabolic activity and virulence response in numerous host tissues [6-8]. Traditionally, nostrils were considered the predominant location of the *S. aureus*, with 20-30% of the adult human. However, it can be found in many skin sites, including the intestine [2-3]. It can infect nearly all host tissue and causes countless apparent skin infections and probably millions of severe infections globally [4-5]. The range of infections are from moderate skin infections such as abscesses, wound infections and furuncles to systemic disease due to epithelial protective layer breaches that can be life-threatening. Besides, due to cuts, *S. aureus* infection also can be found in diabetic patients, which can be life-threatening, and alternative natural based-treatments are critical [9-11].

Aloe vera is a medicinal plant that belongs to the Liliaceae family and has been used in traditional medicine for more than 5,000 years. Due to its high antimicrobial activity, it has been used as a topical medication for skin infections, from cuts to bacterial infections. High antimicrobial activity was observed against gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, also gram-negative bacteria Pseudomonas aeruginosa and Escherichia coli [15-17]. Previously, it was found that its antimicrobial activity can inhibit the growth *of Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium tuberculosis, Streptococcus pyogenes and Salmonella paratyphi* [12-14]. Another traditional medication that is known to have antimicrobial activity is snail mucus.

Chitosan is a polysaccharide with the structure of -(1,4)-2-amino-dioxy-β-D-glucose, which is synthesized by deacetylation of chitin from crustaceans. The applications of chitosan in the medical world include wound healing. The chitosan solution 2% accelerates the wound healing process both macroscopically and microscopically. Many research results report that chitosan has the potential to accelerate wound healing because of its non-toxic, bioactive, biocompatible, antibacterial, antifungal to biodegradable properties [19]. In this study, we would like to investigate the potential natural based-topical treatment for *S. aureus* infection by

combining aloe vera with chitosan and snail mucus as an alternative treatment.

2. METHOD

2.1. Synthesis of Aloe vera Gel Preparations in Combination with Chitosan

Aloe vera stems are washed under running water and separated between the green bark and gel. Next, it is blended and placed in a sealed container [19]. 1.5% chitosan solution (w/v) was prepared with 1% CH_3COOH solution (v/v). For Aloe vera (Av) combined with chitosan (K) gel preparation, 80 grams of aloe vera were synthesized. There are three variations of these combinations, AvK1, AvK2, and AvK3, whose formulation is presented in Table 1.

Table 1. Formulation of the aloe vera gel preparation in combination with chitosan and snail mucus

Materials	Concentration (%)					
	Av:Mucus: K 1:1:1 (AvMK1)	Av:Mucus: K 1:2:1 (AvMK2)	Av:Mukus: K 1:3:1 (AvMK3)	Av	M	K
Aloe vera	10	10	10	30	0	0
Snail mucus	10	20	30	0	30	0
Chitosan 1,5%	10	10	10	0	0	30
Na-CMC	1	1	1	1	1	1
Glycerin	10	10	10	10	10	10
Propylene glycol	5	5	5	5	5	5
Demi water	100	100	100	100	100	100

Na-CMC powder was dissolved in a portion of demi water at 80 ºC then glycerin and propylene glycol were added while stirring [20]. *Aloe vera* gel and 1.5% chitosan solution are added, then snail mucus and demi water is added until it reaches 80 grams and stirred until a homogeneous gel is formed. 1.25% (w/v) NaOH solution is added until it reaches a pH of 6.5-7.0. This treatment is carried out with the same procedure for each variation.

2.2. Organoleptic Test

An organoleptic test was performed to observe aloe vera gel preparations combined with chitosan and snail mucus with several parameters, including color, aroma, and texture.

2.3. Viscosity Measurement

Viscosity (η) is measured using a Hoppler viscometer by calculating the ball fall time (t). The Density (ρ) of the chitosan combination aloe vera gel preparation is obtained by calculating the fluid mass (m), which is the difference in the weight of the picnometer is fully charged with an empty picnometer and divided by the volume of the picnometer (V).

$$\rho = \frac{m}{V} \quad (1)$$

$$\eta = K_{bola}\left(\rho_{bola} - \rho_{fluida}\right) t \quad (2)$$

2.4. Functional Group Cluster Identification

The functional groups of aloe vera gel preparations in combination with chitosan and snail mucus were identified using a Fourier Transform InfraRed (FTIR) spectrophotometer.

2.5. Antibacterial Test of Staphylococcus aureus

All metal tools are sterilized with incandescent flames and cooled [20]. The non-metallic appliance was sterilized using an autoclave (wet heating) at 121ºC for 15 minutes. Nutrient Agar (NA) was prepared from 2.8 grams of NB and 3.5 grams of agar dissolved in 350 mL of demi water. Liquid medium is prepared from 0.48 grams of NB dissolved in 60 mL of boiled demi water. Both media were homogenized and heated by using a hot plate and stirred. Erlenmeyer NA media is covered using cotton swabs and aluminium foil, then autoclaved at 121ºC for 15 minutes. A 10 mL bacterial suspension prepared from 1 mL of *Staphylococcus aureus* stock was added to 9 mL of aseptically cooled NB liquid medium, then incubated at 37ºC for 18 hours. 1 mL of bacterial suspension is put in a petri dish, and 25 mL of sterile liquid NA media is added. The substrate is allowed to stand in the aseptic laminar for 15 minutes until it freezes. 5 mm diameter disc paper is immersed in each sample with the same volume that has been injected and arranged on the media. Incubated at a temperature of 37ºC for 18-24 hours, then measured the diameter of the clear zone formed using a ruler.

2.6. Statistical analysis

Statistical analyses of one-way ANOVA were performed using Prism 7 (GraphPad).

3. RESULT AND DISCUSSION

3.1. Characterization of aloe vera gel in combination with chitosan and snail mucus

Previous research by Susanti & Cahyaningrum 2020 successfully formulated the combination of aloe vera gel with chitosan for its antibacterial potential as a topical medication for skin infection [22]. In this study, snail mucus was added to this formulation in confidence it could add antimicrobial activity to the drug. It was observed that adding 1.5% chitosan with the addition of NA-CMC, glycerine and propylene glycol was effective. These combinations also do not cause irritation and dry skin.

Table 2. Organoleptic observation table from the formulation of aloe vera gel in combination with chitosan and snail mucus

Parameters	Av: Mukus: K 1:1:1 (Av MK)	Av: Mukus: K 1:2:1 (Av MK)	Av: Mukus: K 1:3:1 (Av MK)	Av	M	K
Colour	Yellow	Yellow-brown	Brown	Transparant-green	Brown-green	Clear yellow
Smell	distinctively scented	distinctively scented	distinctively scented	distinctively scented	distinctively scented	distinctively scented
Textures	thick	slightly viscous	slightly viscous	thick +	runny	Thick ++

Organoleptic tests were performed to observe the physical characteristics of the formulation. The higher the concentration of snail mucus, the more distinct the physical appearance compared with the other samples (**Table 2**). It became brownish, slightly viscous and had distinct smells. Viscosity and colour change might be caused by a higher concentration of snail mucus, which has a brownish colour and runny textures. Meanwhile, in lower concentrations of snail mucus, the colour is become more yellow, which might be caused by the colour of chitosan and has thicker textures from aloe vera. A less viscous texture is more favourable because it can help better the application of the gel to the infected skin.

To validate the viscosity observation, each gel formulation was measured by using viscometer. The larger the viscosity value, the gel is thicker and slower the flowing speed. It was observed that the viscosity of aloe vera in combination with snail mucus and chitosan has a smaller viscosity value of around 0.4 mPa.s (**Table 3**). Interestingly, the higher the snail mucus concentration, the viscosity value is hardly increased. This measurement did not correspond directly with the organoleptic observation, in which the higher concentration of snail mucus, the runnier the texture is. However, the viscosity value is similar across combinations, making the viscosity difference between combinations small.

Table 3. Observation of viscosity measurements

Samples	Av:Mukus: K 1:1:1 (AvMK1)	Av: Mukus: K 1:2:1 (Av MK)	Av: Mukus: K 1:3:1 (Av MK)	Av	M	K
Density(g/cm3)	1,8926	1,8933	1,8939	1,8909	1,8943	1,883
Kinds of ball	2	2	2	2	2	6
Ball's Constant (mPa.s cm3/g.s)	0,09	0,09	0,09	0,09	0,09	33
Ball's Density (g/cm3)	2,2	2,2	2,2	2,2	2,2	8,1
Time of ball falling(s)	15,3333	16	16	193,667	16,3333	15,6667
Viscosity (mPa.s)	0,42421107 8	0,441648	0,440784	5,38762227 3	0,4493778	3214,196

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Next, functional group identification was determined by using FTIR. Identification of aloe vera (Av) gel functional groups showed stretching vibrations of O–H and N–H, which were at 3305 cm^{-1} (**Figure 1**). There are C=O stretching vibrations seen in the absorption band of wave number 1631 cm-1, C-H bending vibrations at a wavelength of 1449 cm^{-1}, and C-O stretching vibrations at a wavelength of 1046 cm^{-1}. The functional group of the Chitosan (C) gel was identified as having O–H and N–H stretching vibrations which were seen in the absorption band of 3356 cm^{-1}. C=O stretching vibration of the acetyl group was seen in the band of wave number 1636 cm^{-1} and C-H bending vibration at a wavelength of 1427 cm^{-1}, as well as a C-O stretching vibration at a wavelength of 1040 cm^{-1}.

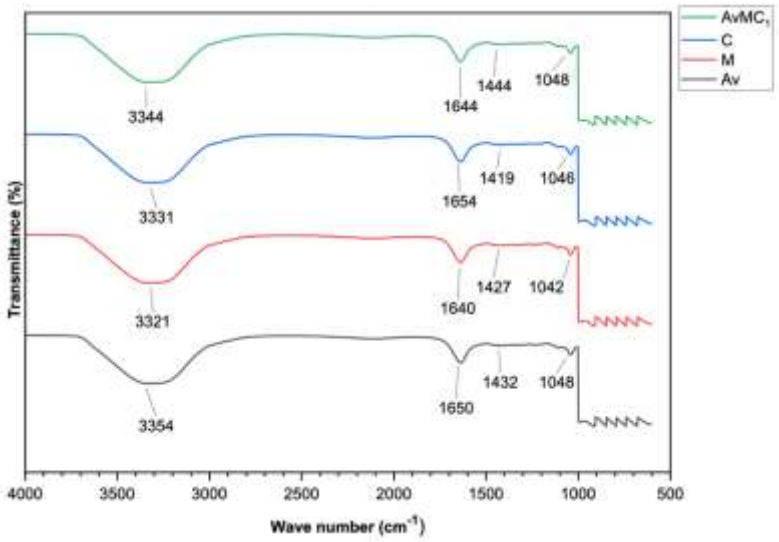

Figure 1. FTIR spectrum from measurements of the aloe vera: snail mucus: chitosan 1:1:1 (AvMC1). Chitosan (C), Snail mucus (M), dan Aloe vera (Av

Meanwhile, AvMC1 gel, a combination of Aloe vera, chitosan and snail mucus, experienced a shift in the FTIR spectra as indicated by the O–H and N–H stretching vibrations at the wavelength absorption band of 3344 cm^{-1}. The C=O stretching vibration of the acetyl group was identified at a wavelength of 1644 cm^{-1}, the C–H bending vibration at a wavelength of 1427 cm^{-1}, and the stretching vibration of C–O at a wavelength of 1048 cm^{-1}. This observation shows that the combination of Aloe vera, chitosan and snail slime showed a shift in the spectra as indicated by the O–H and N–H stretching vibrations at the 3379 cm^{-1}. This characterization shows that the combination of aloe vera, chitosan and snail mucus has a favourable texture for topical medication even though there is a shift in the functional group spectra.

3.2. Staphylococcus aureus anti-bacteria test

Aloe vera and snail mucus were infamous for centuries as antibacterial agents used in traditional medicine. Aloe vera is known to have antibacterial activity and bacteriostatic against *Staphylococcus aureus* as a topical medication from various parts of the plant with a wide range of concentrations. Likewise, snail mucus was also observed to have antimicrobial activity against *S. aureus*. After formulation and characterization of the aloe vera with chitosan and snail mucus, its antibacterial activity is tested using agar well diffusion methods. In this assay, a clear or inhibitory zone around the disc paper will be formed when the substance/drug has antibacterial activity against the tested bacteria. Upon the antibacterial assay, we observed that the equal ratio of chitosan, aloe vera and snail mucus give the largest clear zone (Figure 2). It means that this has the highest antibacterial activity compared with other combinations.

Meanwhile, with the higher ratio of snail mucus, the clear zone that formed is reduced significantly. This observation is unexpected because it was expected that the combination of higher snail mucus concentration would give the most significant inhibitory effect as a synergistic effect of antimicrobial activity from aloe vera and chitosan. However, this observation also shows an optimal concentration/ratio between compositions that would give the most effective antibacterial effects.

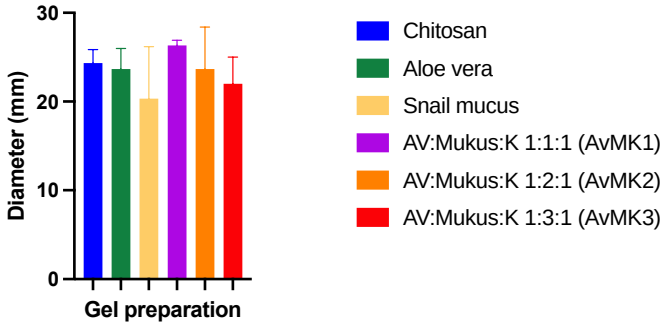

Figure 2. Clear zone observation from the agar well diffusion assays of *S. aureus* with formulation combination. (n=3 for each group, p-value <0.5)

Based on these observations, further research can find the optimal concentration or ratio between the composition to give the best form and most effective antibacterial effects. It is not only crucial for the *S. aureus* infection due to cuts but also for a skin infection caused by diabetes. Skin and soft tissue infections (SSTIs) are one of the skin infections caused by S. aureus often found in the limbs of diabetic patients. It could occur from mild to life-threatening infections, and sometimes amputations of the limbs are necessary to prevent a higher chance of morbidity. It becomes crucial to find a better natural-based medication for skin infections, especially those caused by *S. aureus*, as an alternative to chemical-based medicines.

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