Multidisciplinary Applied Research and Innovation Vol. 3 No. 5 (2022) 160-168

MARI

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/mari
e-ISSN:2773-4773

Allicin Infused Virgin Coconut Oil with Anti-bacterial Potential

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DOI: https://doi.org/10.30880/mari.2022.03.05.024
Received 15 July 2022; Accepted 30 November 2022; Available online 31 December 2022

Abstract : The production of allicin infused soap was aimed to be an alternative to commercial available medicated soaps which contain high in chloroxylenol. High chloroxylenol content is toxic and harmful which may be lethal to humans at high concentration. Synergistic effect of allicin and virgin coconut oil (VCO) are contributing to the anti-bacterial activity of the formulated soap. The extraction of allicin was done by using ultrasonication method. The extracted allicin was infused in the VCO and undergo saponification process to produce the soap. The performance of the soap were determine through its physical, chemical and biological properties. Method that have been used to determine its physical properties are foam stability test and sensory evaluation test. For chemical properties, moisture content, pH and free alkali content test were done whereas anti-bacterial property of the soap was executed using the disk diffusion test to study its biological characteristic. The disk diffusion test was done on gram-negative and -positive bacterial which were *Escherichia coli (E. coli)* (ATCC 25922) and *Staphylococcus aureus (S. aureus)*(ATCC 25923) respectively. For its physical property, it was found that S1 have good foam stability (98.11%). In terms of sensory preference, it was found that S1 smell is unfavourable as it contains allicin originated from garlic. The chemical analysis found that S1 have alkaline pH (9.98) and contain sufficient amount of free alkali (0.861%) and was within the recommended range. The S1 sample has low moisture content (15.4%) due to its high VCO content. Interestingly, the addition of allicin in S1 increased its anti-bacterial effect on *E.coli* as the formation of inhibition zone (11.5 mm) was 10 times larger compared to S2. However, S1 was found to be less potent to *S. aureus* as the inhibition zones formed was smaller (1.2 mm). Based on the obtained result, it is concluded that allicin infused soap is a potential substitute to readily available anti-bacterial soap with comparable physical, chemical and biological properties.

Keywords: saponification, allicin, virgin coconut oil, bacterial inhibition

1. Introduction

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Allicin is a sulfur-containing natural compound with many different biological properties which responsible for the typical smell and taste of freshly cut or crushed garlic. In nature, allicin is produced after the disruption plant tissue due to enzymatic reaction. Allicin is also a compound that is responsible for the antibiotic properties in garlic. Previous study reported that the synergy of allicin extracted from garlic with cysteine were used in the development of antibiotic to inhibit bacterial activity. The mechanistic of allicin antimicrobial action happens intracellularly as allicin will penetrate into the intercellular cavity of the microbes and reacting on its vital organelle thus lead to cell inactivity or death [1].

There were several investigation held that proved the allicin anti-bacterial activity against gram-positive and gram-negative bacteria depends on the amount of allicin [1]. Borlinghaus et al. (2014) [1], stated that allicin content in garlic are able to shrivelled acne and cure boils. Other than that, it can reduce blackhead and whitehead on the skin. In addition, garlic have high level of antioxidant that can help in preventing the free radical damage for example it can cure skin inflammation and irritation. Virgin coconut oil (VCO) contains high lauric acid content (46-50%) attached to glycerol backbone to form trglycerides. Mainly, lauric acid is active as anti-bacterial, antiprotozoal and antiviral component. Lauric acid is proven to be more active anti-bacterial agent compared to other acids due to high content [2]. VCO traditionally has been used as moisturizer since the past study showed that VCO has skin protective properties. VCO is capable of suppressing Lipopolysacharrides induced pro-inflammation cytokine in human monoocytic leukemia cells. VCO also could be useful in treating skin disorder with permeability barrier dysfunction, especially those accompanied by reduce epidermal protein expression such as atopic dermatitis and eczema [1].

There are a lot of soaps produce using chemicals readily available in the market. Medicated soap especially contains chloroxylenol which act as anti-bacterial agent. However, excessive exposure to chloroxylenol has adverse effects including lethal due to its toxic behaviour. Medicated soap also kills good bacterial species which guards the skin safety from the infestation of harmful pathogenic bacteria thus leaving the skin more vulnerable especially to bacterial causing acne [3]. The objective of this study is to saponify allicin infused VCO as a potential alternative to commercially available soap used to treat skin conditions related to bacterial infestation that are from natural sources, safer and non-toxic for cosmeceutical application. The synergistic effects of allicin and VCO may provide an alternative to medicated soap with both anti-bacterial and moisturizing properties that is suitable to be used daily. Due to its antioxidant capability with high acid and allicin content in VCO and allicin may help in inhibiting bacterial growth on the skin surface. At the same time, VCO are able to provide good moisturizing properties which reduce excessive sebum production consequently helps in managing the formation of blackheads and whiteheads soothing the skin upon application. Lack of sufficient moisturization may lead to oily skins which may clog the pores making skin to be prone to infection, inflammation and acne [4]. The performance of the soap will be investigated through its physical, chemical and biological properties. Method that have been used to determine its physical properties are foam stability test and sensory evaluation test. For chemical properties, moisture content, pH and free alkali content test were done whereas anti-bacterial property of the soap was executed using the disk diffusion test to study its biological characteristic.

2. Materials and Methods

2.1 Materials

Virgin coconut oil (VCO) and garlic was purchased from a local supermarket. The saponification process and the determination its chemical and biological activitird involved the use of sodium hydroxide (NaOH) (Merck, Germany), stearic acid (Merck, Germany), sodium chloride (NaCl) (Merck, Germany), ethanol (Merck, Germany), glycerin (Merck Germany).phenolphthalein (Merck, Germany),

potassium hydroxide (KOH) (Merck, Germany), nutrient agar (Merck, Germany). All reagents used were of analytical grade. Equipment used in this study were ultrasonic bath (GTsonic, VGT 1990QTD, China), centrifuge (Esco, TCV 1500, Singapore), stiring hotplate (Favorit, HS0707V2, Korea). Micropipette (HWLAb, China), Bunsen burner (Hmbg, Italy), pH meter (Mettler toledo, EL20, Singapore) sterile petri dish (Brandon, Germany), autoclave (Systec, 1295, Germany), oven (Memmert, UN75, Germany), incubator (Esco, IFA- 54-8, Singapore) and anti-bacterial (Pensonic, PB3202, Malaysia) filter paper (Whatman, No. 1, United Kingdom).

2.2 Etraction of Allicin from Garlic

A 5 g of garlic were sliced into fine equal pieces and mixed with 50 ml of deionised water. The mixture was placed in an airtight container. The sample was placed in sonicator (GTsonic, VGT 1990QTD, China) for 30 minutes at 30°C. After sonication, the sample was separated from impurities by centrifugation at 3000 g for 2 minutes. After that, the solution was filtered by filter paper (Whatman, No. 1, United Kingdom to remove the undissolved garlic. The filtered sample then was stored at 4°C [4].

2.3 Saponification of Allicin infused VCO

A 5 g of stearic acid was melted at 80°C followed by the addition of 30 g of VCO. The mixture was heated and stirred by using stirrer (Favorit, HS0707V2, Germany) at temperature 80°C for 5 minutes. A 0.2 g of NaCl were added and stirred for 5 minutes. Next, 0.33 g/mL of NaOH solution was added into the mixture and stirred until it thickens. A 5 g of glycerin were added stirred homogeneously in 5 minutes until fully dissolved. The temperature of mixture is lowered to 25°C to add 5g of allicin extract and is lableled as S1. Soap with no allicin serves as a negative control (Labeled S2).The mixture was poured into mold and after 10 minutes, the soap sample was covered with aluminium foil and stored at ambient temperature for 24 hours to hardens. **Table 1** showed the composition S1 and S2 soaps. Soaps were stored in dry condition at room temperature before further analysis.

Table 1: **Formulation of Allicin infused VCO Soaps**

Raw Materials (%)	Samples		
	S1	**%**	**S2**
1. Virgin Coconut oil (VCO)	30.0	59	30.0
2. Sodium Hydroxide (NaOH)	5.0	9.9	5.0
3. Stearic Acid	5.0	9.9	5.0
4. Sodium Chloride (NaCl)	0.2	0.4	0.2
5. Glycerin	5.0	9.9	5.0
6. Allicin Extract	5.0	9.9	-

3.3 Physical Properties of Soap

3.3.1 Foam Stability

The foam stability of the soap was done by grinding 5 g for soap samples (S1 and S2) using a dry grinder (Pensonic, PB3202, Malaysia). The ground soaps were dissolved in 100mL of distilled water and were stirred by using magnetic stirrer (Favorit, HS0707V2, Germany) for 15 minutes. The mixture was poured into a measuring cylinder as shown in **Figure 1**.

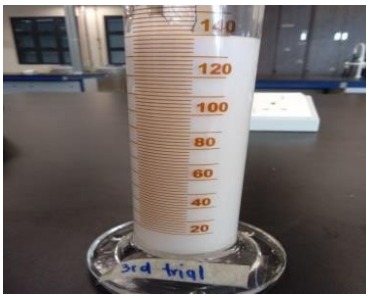

Figure 1: The settling of foam for S1 and S2

The height of foam produced were measure (initial height). The mixture was let to settle for 15 minutes and the height of the foams were measured (final height) [5]. The percentage of foam stability was calculated using the **Equation 1**:

$$\text{Percentage of foam stability (\%)} = \frac{\text{Final height of foam (mL)}}{\text{Initial height of foam (mL)}} \times 100 \qquad \textbf{Eq. 1}$$

3.3.2 Sensory Evaluation Test

A sensory survey was conducted on 30 UTHM students around Pagoh Residential College which consist of 15 males and 15 females. A questionnaire was distributed to the respondents to determine the preferences on the colour, aroma, hardness, foam stability and moisture feel of the produced soaps (both S1 and S2 samples). Respondents were to rate their preferences according to the given scale of 1 to 5 in which scale 1 is being extremely dislike and 5 as the exteremly like. The criteria of the survey is tabulated in **Table 2**.

Table 2: Criteria of Soap Sensory Survey

Criteria	**Level of Preference/ Scale**				
	Extremely dislike	**Dislike slightly**	**Indifferent**	**Like slightly**	**Exteremly like**
1. Colour 2. Aroma 3. Hardness 4. Foam Stability 5. Skin moisture feel	1	2	3	4	5

3.4 Chemical Properties of Soap

3.4.1 Soap Moisture Content

The mositure content of the soap was determine by grinding 5 g for soap samples (S1 and S2) using a dry grinder (Pensonic, PB3202, Malaysia). The samples were spread evenly in a drying pan and were dried at 105˚C until a constant weight was achieved [5]. The percentage of water content was calculated using **Equation 2**.

$$Percentage\ of\ water\ content\ (\%) = \frac{Weight\ of\ dried\ sample\ (g) - weight\ of\ sample(g)}{weight\ of\ sample\ (g)} \times 100 \qquad \textbf{Eq. 2}$$

3.4.2 Soap pH Determination

The water content of the soap was determine by grinding 5 g for soap samples (S1 and S2) using a dry grinder (Pensonic, PB3202, Malaysia). The ground soap was dissolved in 100mL of distilled water. The pH electrode (Mettler toledo, EL20, Singapore) was rinsed with distilled water then dried with paper towels The pH electrode was dipped into mixture of soap (as in **Figure 2**) and distilled water until the value of pH became constant. The value of pH was recorded. [5].

Figure 2: Experimental Setup for pH Analysis on Both S1 and S2 Samples

3.4.3 Free Alkali Content

The free alkali content of the soap was determined by using tirtration method. A 5g of ground soap samples (S1 and S2) was dissolved in a boiling ethanol containing phenolphthalein indicator of 100:1 mL ratio. The mixture was cooled and titrated with 0.1 N KOH until the colour of the mixture changes to pale purple as shown in **Figure 3**. The volume of KOH used were recorded [5]. The percentage of free alkali content was calculated using **Equation 3**.

$$Free\ Alkali = \frac{volume\ of\ KOH\ (ml) \times concentration\ of\ KOH\ (N) \times 0.205}{weight\ of\ sample\ (g)} \qquad \textbf{Eq. 3}$$

Figure 3: The colour of ethanol: phenolphtalene changes after titration with KOH to determine free acid content in S1 and S2

3.5 Biological Properties of Soap

3.5.1 Preparation of Microbial cell suspension and Nutrient Agar

A 28g of nutrient agar was dissolved in 1L of distilled water. The media was autoclaved (Systec, 1295, Germany) at 120˚C. The sterile media is kept at 60˚C in the oven (Memmert, UN75, Germany) to prevent it from solidifying. The microbial cell suspension of gram-positive and gram-negative bacteria which are *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) and *Escherichia coli* (*E. coli*) (ATCC 25922) respectively was prepared. A loopful of bacterial cells activated at 37˚C for 24 hours is transferred in 1mL of sterile distilled water and was mixed until fully dissolve. A 1mL of the microbial suspensions (*S. aureus* and *E. coli*) was mixed with 20mL of nutrient agar asceptically and was mixed thoroughly. The agar containing cell suspension was poured into a sterile petri dish and left to solidify [5].

3.5.2 Disk Diffusion Assay

Disk diffusion method was used to analyze the anti-bacterial properties of soap samples (S1 and S2). Preparation of disk impregnated with S1 and S2 was done by dissolving 5g of ground samples in 50mL of sterile distilled water. A sterile disks made of punctured filter paper (Whatmann, No.1, United Kingdom) were submerged in the dissolved sample. Excess samples on the surface of the disk was discarded. The disk diffused with the sample was placed on the surface of the agar in the prepared petri dish (in subsection 3.5.1). The plates were incubated (Esco, IFA- 54-8, Singapore) at 37°C for 24 hours. The inhibition zones formed after incubation were measured and recorded [5].

4.0 Result and Discussion

4.1 Physical Properties of Soap

The stability of foam for sample S1 and S2 is tabulated in **Table 3**.

Table 3: Foam Stability Analysis of S1 and S2 Samples

Sample	Initial Height (mL)	Final Height (mL)	Foam Stability (%)
S1	106	104	98.11
S2	94	92	97.87

Based on the obtained data, it was found that both S1 and S2 have good foam stability. The addition of glycerin in the soap formulation may be the contributing factor to this property as it was proven to improve lather ability in soap [6]. The formation of foam reduces the surface tension of water thus making it able to effectively remove sebum or oil from the skin's surface. The durability of the foam produced also indicates that both VCO and glycerin acts as foaming and surfactant agent. Both of glycerin and VCO was also reported to act as an emollient which gives out the moisturizing effect of the soap [6].

The sensory evaluation test was conducted to determine the performance of the soap based on the colour, aroma, soap texture (hardness), foam stability and skin moisture feel for both S1 and S2 are shown in **Figure 4(a)** and **4(b)** respectively.

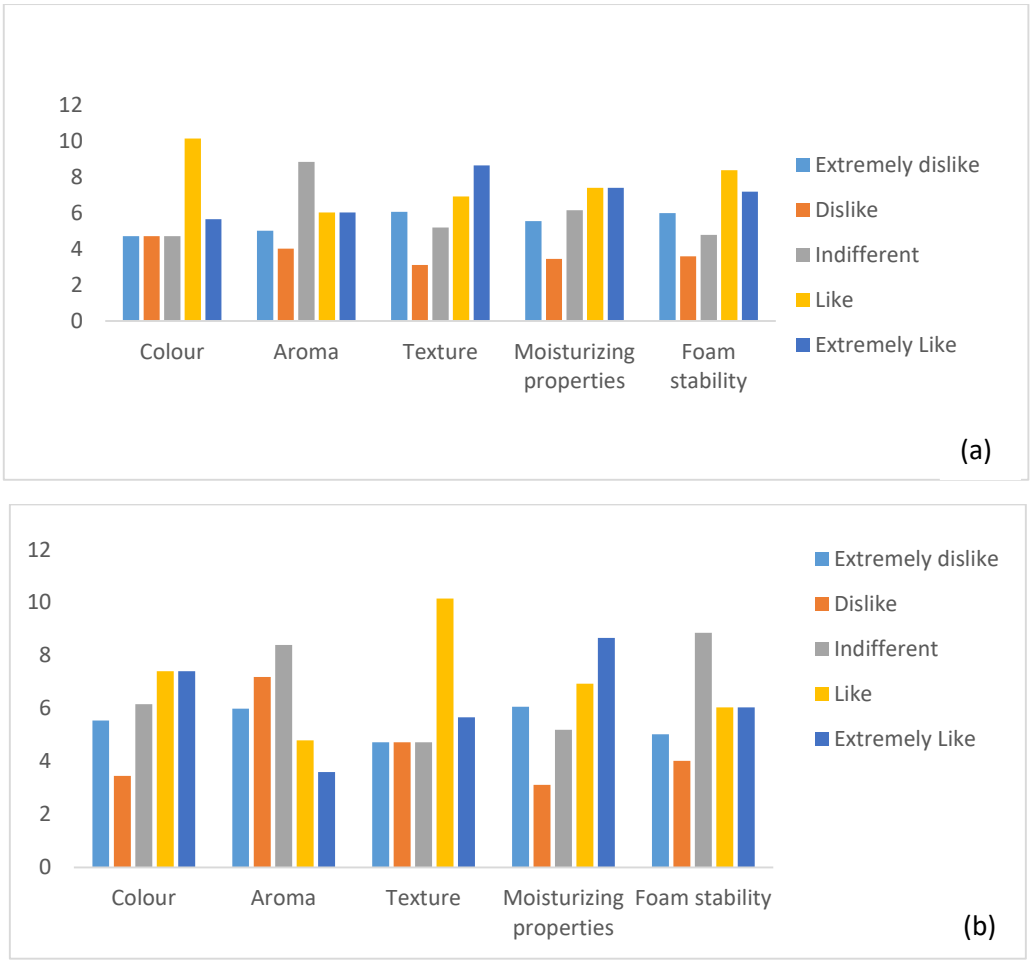

Figure 4: Sensory Preferences of Respondents from UTHM Pagoh Towards (a) S1 and (b) S2 Samples

Refering to **Figure 4**, it is concluded that the colour of S2 is higher because the soap is clearer meanwhile sample S1 has white opaque colour due to the addition of allicin extract. The aroma for S2 is more preferable as S1 has stronger smell due to the addition of allicin. For the hardness, both S1 and S2 has similar hardness as both have the same composition of VCO and glycerin content. The addition of allicin in S1 has no effect on its building structure. Both S1 and S2 has high foam stability. It was also observed that both S1 and S2 is preferable as it left an afterwash moisturizing effect after application.

4.2 Chemical Properties of Soap

The chemical properties of S1 and S2 samples are as tabulated in **Table 4.**

Table 4: Chemical Properties of S1 and S2 Samples

Sample	Moisture Content (%)	pH	Free Alkali (%)
S1	15.4	9.98	0.861
S2	13.5	9.69	0.984

According to **Table 4**, the moisture content of all samples exceeded 11.98% which are of suitable range for soap products. However, the moisture content is considered low in value. The low percentage of moisture content indicates that the S1 and S2 are low in water content. The low water content also signifies that both S1 and S2 contains high fatty acid content which is contributed by the high percentage of VCO used in the formulation. High fatty acid content provides good moisturizing effects which is agreed by the respondents during the sensory evaluation as discussed in section 4.1. Low water content also helps in prolong the duration of storage as it provides an unconditional environment for bacterial growth. Interestingly, despite of its high fatty acid content, the pH of S1 and S2 are in alkaline range. It was also found that the pH value of S1 and S2 are ranging between 9-10.5 which are suitable for skin [7]. The addition of allicin in S1 sample caused the slide increment in its moisture content. The alkalinity of the soaps were due to the addition of NaOH during the saponification process. It was also proven that the soap also contains high free alkali content which supports the alkaline pH values of both S1 and S2 samples.

4.3 Biological Properties of Soap

The formation of inhibition zones on the agar signifies that inhibition of bacterial growth has occured. The diameter or size of the inhibition zones formed indicates the degree of the potent effect of S1 and S2 sample on the bacterial growth (as shown in **Table 5**).

Table 5 Anti-bacterial Activity of S1 and S2 on *E.coli* and *S.aureus* Strains

Sample	Inhibition zone (mm)	
	E.coli **(ATCC 25922)**	***S.aureus*** **(ATCC 25923)**
S1	11.5	1.1
S2	1.35	1.2

Based on **Table 5**, both S1 and S2 exhibit anti-bacterial activity as inhibition zones on both plates containing *E.coli* and *S. aureus* were observed which is contributed by the antioxidant and anti-bacterial property possessed by the VCO. It is also noteworthy that the S1 produced stronger anti-bacterial effect on the growth of *E. coli* compared to S2. This proved that the allicin present in S1 enhanced the anti-bacterial properties of the soap samples and is a powerful anti-bacterial agent against gram-negative bacteria. Both S1 and S2 samples have lower anti-bacterial capacity against *S. aureus* strain which indicates that it is a weak growth inhibitor for gram-positive strains.

5.0 Conclusion

It was proven that the formulated soap infused with allicin are able to inhibit the growth of both gram-positive and gram negative bacteria. Moreover, it chemical properties was found to be suitable to be used on skin as the pH and free alkali content matches the recommended range. The allicin infuced soap also cleans well as it has good foam stability which guarantees that it can remove oily particles effectively. The high content of VCO and the addition of glucerine helps in providing good moisturizing characteristics of the allicin infused soap. Based on the findings, it can be concluded that allicin infused VCO soap is a potential alternative to the comercially available anti-bacterial soap with moisturizing effect.

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