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Formulation And Evaluation of Sunscreen Lotion Incorporated with *Centella Asiatica* Extracts

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Abstract: The compounds that exist in the sunscreens nowadays have come in some health effects like rashes, acne, crusted rashes and in order to tackle these side effects, natural or herbal sunscreens comprising of organic material are made. This research aims to get the right sunscreen formulation incorporated with Gotu Kola or *Centella asiatica* extract in sunscreen, understanding the physicochemical characteristics, and acquire information about the formulations that has been produced by evaluating its physicochemical properties, SPF value, blocking ability, antimicrobial and antioxidant activity. In this study, preparation of sunscreen base and incorporation of *C. asiatica* extract was conducted to produce the formulated sunscreen, followed with its evaluation tests, which involves DPPH assay for antioxidant and Disc Diffusion method for antimicrobial testing. Sunscreen with formulation F20 (incorporated with 20% of *C. asiatica* extract) was seen to have a stable pH value of 7.25 ± 0.012. Next, the sunscreens were evaluated using SPF analysis. F20 produced has a maximum SPF value of 22.21. To evaluate the sunscreen properties, the ability of sunscreen to block UV light to microbes, Antioxidant and Antimicrobial activity of sunscreen was done. The inhibition zone of the herbal sunscreen containing *C. asiatica* with 20% concentration is the highest which is 16mm at 100% concentration. The study showed that increasing *C. asiatica* extract led to a better result in the antioxidant activity in DPPH radical scavenging assay. The data shows suggests that sunscreen F20 formulated with 20% concentration of *C. asiatica* extract is suitable to be produced and commercialized.

Keywords: Herbal Sunscreen, *Centella Asiatica*, UV Filter, Sun Protective Factor

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1. Introduction

The skin is the biggest part of the body and has approximately sixteen percent of the human body weight. Skin serves as a major role to protect human body from numerous environmental effects such as the UV rays. Sunscreens have been recommended as a form of protection against sunlight, with protection increasing with higher sun protection factor. Sun Protective Factor, or SPF is the ratio of UV energy needed to create a minimal erythemal dose (MED) in protected skin to unprotected skin, is typically used to measure the efficacy of sunscreens [1]. Inorganic filters provide broad-spectrum protection, photostable, generally safe or non-irritant, and dispersions are easy to incorporate into product bases. Some disadvantages of inorganic UV filters are that they have a perceived poor aesthetic feel due to their white cast, and in their powder form, they can be difficult to formulate with.

Therefore, in this study, the organic sunscreen with herbal properties will be formulated and evaluated. The extract from *Centella asiatica* will be incorporated in sunscreen formulation. *Centella asiatica* is a herb that has high potential to be incorporated into sunscreen formulation due to its antioxidant, anti-inflammatory and other benefit properties. It was expected that as the concentration of the extract increases in the sunscreen, the better the results.

1.1 Herbal Sunscreen

Due to environmental, health concerns and safe usage, researchers have recently focused on developing sunscreens with broad spectrum anti-UV radiation efficiency with low concentrations of chemical UV filters and bioactive compounds. According to Chanchal (2008), herbal extracts provide a healing, softening, regenerating, and sunscreen action on these areas [2]. Numerous plant-based substances have been found to be nontoxic, antimutagenic, and anticarcinogenic and have the power to dramatically block a wide range of cellular processes at different stages of carcinogenesis.

1.2 *Centella Asiatica*

Centella asiatica is commonly known as Indian Pennywort, belongs to the family Apiaceae [3]. *C. asiatica* is one of the herbs for treating skin problems, to heal wounds revitalizing the nerves and others. The main active ingredients for *C. asiatica* are Asiatic acid and Asiaticoside, where both of them belong to triterpenoid saponins [4]. It has inhibitory effect on many kinds of bacteria. Asiatic Acid and Asiaticoside can promote wound healing, and can be used to repair skin scars while accelerating the synthesis of fibroblasts and collagen. It can reduce skin hardness, improve skin nutrition, and facilitate skin metabolism [5].

2. Materials and Methods

This section reviewed on the materials and the experimental procedures that was conducted in this study, starting from formulation of sunscreen, incorporation of C. asiatica in sunscreen and all the tests done on the lotion produced. The tests included SPF analysis, antioxidant, antimicrobial and several physicochemical tests including pH evaluation, stability test.

2.1 Materials

The details of chemical and reagent will be used in this project are listed below. The chemical usage is divided between its sunscreen formulation and other usage in the experiments.

- Stearic acid
- Liquid Paraffin
- Glycerin
- Triethanolamine (TEA)
- Metyl Paraben
- 99% Methanol
- Ethanol

- Mueller Hinton Nutrient Broth
- 1,1-diphenyl-2- picrylhydrazile (DPPH)
- Cetyl Alcohol

The details of apparatus used in this project are listed below.

- Analytical Balance
- HANNA pH meter
- UV VIS spectrophotometer
- Autoclave
- Incubator
- Beaker
- Petri Dish
- 10 – 1000µL micropipette
- Hot Stirrer Plate

2.2 Methods

The production of sunscreen formulation was divided into two parts: the oil phase (phase A) and the water phase (phase B). Five sunscreens were formulated using the combination of phase A and phase B through melting of ingredients at 65-70℃ for 30 minutes. The sunscreen lotion bases were then incorporated with accurate amounts of *C. asiatica* extract according to formulation in Table 1. The determination of its properties were made after the production of sunscreens [6].

Table 1: Composition for formulation of sunscreens incorporated with *C. asiatica* extract

Ingredient	Formulation (%)				
	F0	F5	F10	F15	F20
Stearic Acid (mL)	2.5	2.5	2.5	2.5	2.5
Liquid Paraffin (mL)	7	7	7	7	7
Cetyl Alcohol (mL)	0.5	0.5	0.5	0.5	0.5
Glycerin (mL)	5	5	5	5	5
Metyl Paraben (mL)	0.05	0.05	0.05	0.05	0.05
TEA (mL)	1	1	1	1	1
Distilled water (mL)	83.95	78.95	73.95	68.95	63.95
Centella asiatica Extract (mL)	0	5	10	15	20

*F0, F5, F10, F15 and F20 indicates sunscreen formulation with different amount of *C. Asiatica* Extract

Physicochemical parameter

Two types of physicochemical testing such as pH and stability test were performed for the sunscreen formulations before proceeding to evaluation of sunscreen.

pH

The pH measurement was carried out using HANNA pH meter. The HANNA tool was calibrated first using 3 calibration unit buffer before being dipped into the sunscreen for readings. The electrode was dip into the solution and let move until the position of number is constant. The probe was cleaned using distilled water after every usage [6].

Stability test

The lotion sample was put into a container and spread evenly and the homogeneity of the sunscreen was observed. The time and temperature of the environment was taken into consideration when leaving the sunscreen out. The lotion was considered homogenous if there are no separation between oil phase and water phase after 48hr and 1 week [7].

SPF analysis

A 200mg of each formulated sunscreen was weighed and transferred to a 100 mL volumetric flask. The sunscreens were diluted with ethanol and followed by vigorous vertexing. The absorption spectra of samples in solution can be obtained in the range of 290 to 320 nm using 1 cm quartz cell, and ethanol in ethanol as a blank. The absorption data can be obtained in the range of 290 to 320 (the range of UVB) every 5 nm interval [8]. These absorbency results are then used to determine the SPF values using formula for SPF value presented in the Equation 1.

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times 1(\lambda) \times \mathrm{Abs} \quad \text{Eq. 1}$$

Where CF is the correction factor (10), EE (λ) x 1 is the erythmogenic effect of radiation with wavelength λ and Abs (λ) is the spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants [8].

Blocking ability of sunscreen

In this method, nutrient agar media plates were seeded *with E. coli*. Five Petri dishes was labelled and divided half by drawing a line on the bottom half of the plate. One side was labelled "C" for control and the other side with the sunscreen. Half top of the Petri dish was covered with the parafilm and folded to the beneath the bottom of the Petri dish. 0.5g of different sunscreen formulation was weighed for each Petri dish. The sunscreen was applied evenly to the parafilm using the same technique for each sunscreen [9]. Using the sunlight as UV light, the Petri dishes were exposed to the light for 30 minutes to make sure dishes are same distance from light source. Once samples have been exposed to UV light, incubate the petri dish for 24hrs. Following 24hours growth, the number of bacterial colonies of *E. Coli* growing on the Petri dishes was determined [9].

DPPH scavenging assay

1M of DPPH was prepared by weighing 4mg of DPPH and mix well with 100 ml of 99% methanol. The flask was shake to homogenous the solution. The flask was sealed with aluminium foil and kept in cool condition. For preparation of sample, the previous method was followed. 1g of each sample was mixed well with 10 ml of distilled water. Each of samples was prepared with different concentration. From each sample concentration, 1ml of solution was mixed with 6 ml of methanol and 3ml of DPPH solution. The control solution was prepared by replacing the samples solution with distilled water. Vortex the mixture to get the homogenised solution before keep it in the dark for 30 minutes. Then, calculate the absorbance of the samples by using UV-vis spectrometer assay at 517 nm wavelength [10]. The percentage of scavenging assay can be calculated by using Equation 2.

$$\text{Radical Scavenging } \% = \frac{\mathrm{A_{control}} - \mathrm{A_{Sample}}}{\mathrm{A_{control}}} \times 100 \quad \text{Eq. 2}$$

Where, $\mathrm{A_{Sample}}$ is absorbance of the extract and $\mathrm{A_{control}}$ is absorbance of control [10].

Disc diffusion susceptibility method

In this method, nutrient agar media plates were seeded *with S. epidermidis and E. coli*. The bacteria were sub culturing for 18-24 hours before used. Whatman No. 1 filter paper disc with size 6mm in diameter was impregnant with 20 ul of different concentration of samples. The positive and negative controlled used was Ampicillin and distilled water respectively. The disc was placed on the surface of inoculated and dried plate by using sterile forceps [11]. Media plates were incubated at 37℃ for 18-24 hours. The samples diffused from the disc into the agar media would prevent the growth of microorganism around the disc if it susceptible. The zone of inhibition was measured the next day of each treatment.

3. Results and Discussion

The results obtained on F20, a higher concentration of *C. asiatica* incorporated in the sunscreen have better properties than the lower concentrations through the tests conducted. *C. asiatica* have shown that their bioactive components are anti-oxidant, antibacterial and are able to act as a UV blocker. Overall, SPF analysis, DPPH free radical scavenging activity, and antimicrobial activity tests were performed to confirm the sunscreen formulation incorporated with *C. asiatica* extract are able to perform as a UV filter for sunscreens.

Analysis of physicochemical properties

The results below show the results for physicochemical test which is pH test and Stability test.

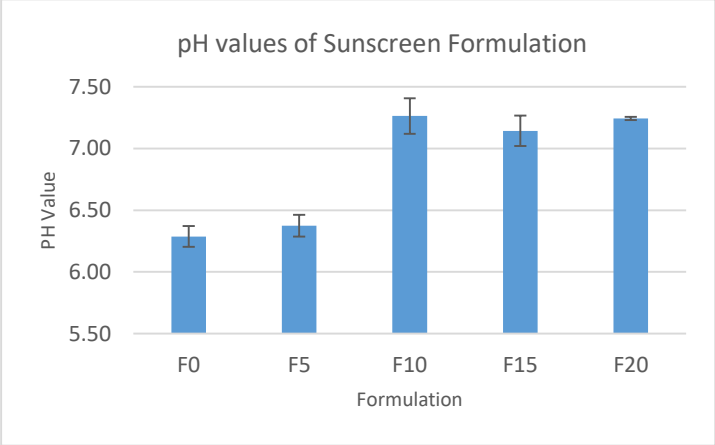

Figure 1: pH values of sunscreen Formulation, n=5. F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract

Table 2: Variables in consideration for Stability testing observations

Time	Temperature	Condition
0hr	40℃	Closed, dark container
48hr	28℃	Closed, dark container
168hr	29℃	Closed, dark container

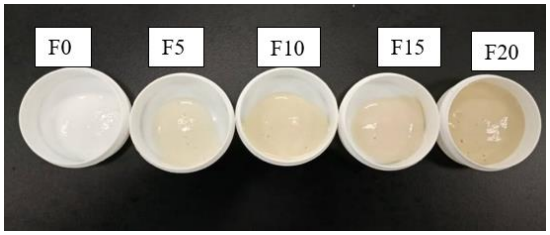

Figure 2: Observation on sunscreen after 48hr placed at room temperature. F0: sunscreen without C. asiatica extract, F5: sunscreen incorporated with 5% concentration of C. asiatica extract, F10: sunscreen incorporated with 10% concentration of C. asiatica extract, F15: sunscreen incorporated with 15% concentration of C. asiatica extract, F20: sunscreen incorporated with 20% concentration of C. asiatica extract

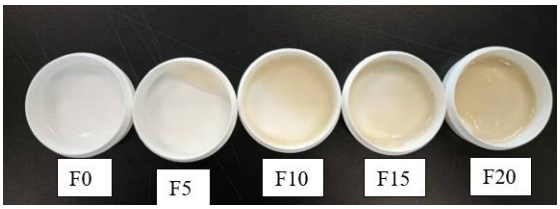

Figure 3: Observation on sunscreen after 1 week placed at room temperature. (F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract)

The pH results in Figure 1 shows that as the concentration of *C. asiatica* increased, the pH value of the formulated sunscreen increased on 10% concentration of *C. asiatica* extract. The pH stabilizes at 7 pH value on F10 to F20, which is within the typical pH range of 5-9.5 according to the SASO 1512/2022 [12]. All sunscreen formulated had a pH value within the specified range, implying that they were safe for application on the skin. The pH of creams was determined to examine the possible side effects due to acidic or alkaline pH, which can lead to irritation of skin. Acidic or alkaline pH may cause irritation to the skin and influence the rate of hydration of polymer [12]. From Figure 2 and 3, it shows that there is no separation or colour changes on the sunscreens. This shows that the sunscreen undergoes no physical changes after being left in a certain amount of time and temperature.

Analysis of SPF (Sun Protective Factor)

According to FDA, the SPF value indicates the level of sunburn protection provided by the sunscreen product. The effectiveness of a sunscreen is usually expressed by sun protection factor (SPF) which is the ratio of UV energy required to produce a minimal erythemal dose (MED) in protected skin to unprotected skin [8]. The main way to assess how well a sunscreen composition works is by measuring the SPF rating. This formulation aimed to produce a sunscreen that could reach a minimum Sun Protection Factor of 15 for UVB sunscreen at 290nm-320nm wavelength. Table 3 shows the results taken from UV-vis at 5nm interval from wavelength 290 to 320. Table 3 shows the absorbency taken for every 5nm wavelength for every formulated sunscreen.

Table 3: UV-vis spectroscopy data result of sunscreen formulations incorporated with *C. asiatica* Extract

Wavelength	EE x 1	Formulation (%)				
		F0	F5	F10	F15	F20
290	0.015	0.754	0.816	0.842	0.871	0.877
295	0.0817	0.767	0.820	0.690	0.869	0.877
300	0.2874	0.754	0.810	0.762	0.771	0.880
305	0.3278	0.894	0.879	0.890	0.836	0.877
310	0.1864	0.860	0.879	1.103	0.817	0.917
315	0.0837	0.717	0.880	1.159	1.091	1.012
320	0.0180	0.717	0.880	0.867	1.091	0.913

*F0, F5, F10, F15 and F20 indicates sunscreen formulation with different amount of *C. asiatica* Extract. F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract, EE X 1: Erythmogenic effect value of every 5nm wavelength.

Table 4: SPF Value at 295nm

Formulation	SPF value
F0	19.54
F5	20.76
F10	17.48
F15	22.00
F20	22.21

*F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract

Based on Table 4, it can be seen that sunscreen F20 produces the highest SPF value at 295nm which is 22.21. It can also be seen that most of the formulation reached the UVB spectrum SPF value at minimum of SPF 15 and nearly all the other concentration has SPF values that are equal to or nearly identical to those determined using the Mansur equation [13]. The SPF value above was taken at 295nm wavelength and as the wavelength increased, the SPF value increased.

Analysis of blocking ability of sunscreen to block UV light to *Escherichia Coli*

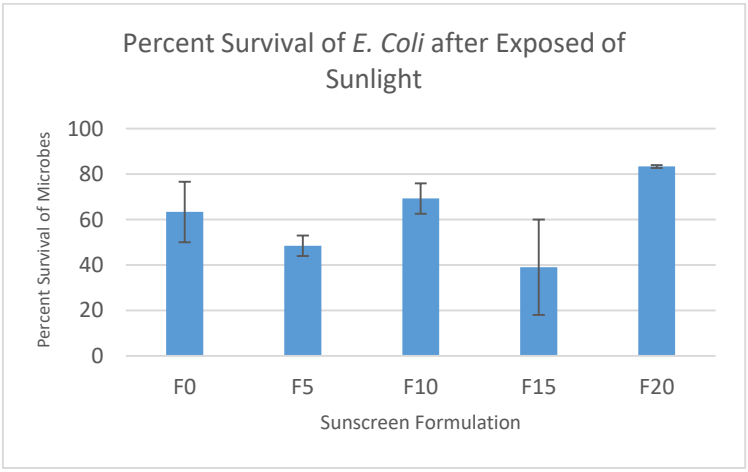

Figure 4: Percent Survival of *E. Coli* after Exposed to Sunlight (F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated

with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract)

Figure 4 shows the graph bar results from the blocking ability of sunscreen when bacteria was exposed to UV light. The number of colonies represents the bacteria that survived the UV light and reflects the amount of protection the sunscreen offered. From the table, it can be seen that F20 has the highest percent survival of *E. coli* bacteria which is 84±0.63% and the lowest is F15, 18±20.1. Theoretically, the colony count on sunscreen should be higher than that in control as it indicates that the bacteria is protected from the UV light using the sunscreen but F15 result shows the opposite [15]. However, some results could be proven that as the concentration increased, the bacteria survived more than that of in low concentration.

Analysis of antioxidant activity

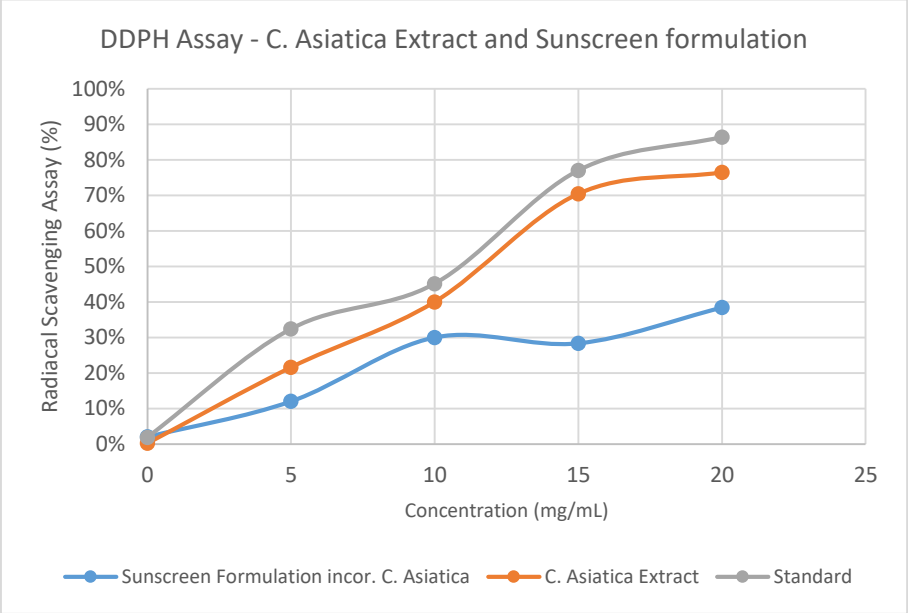

Figure 5: DPPH Assay on 0, 5, 10, 15 and 20mg/mL concentration of *C. asiatica* Extract, Sunscreen Formulation incorporated with C. Asiatica Extract in different concentration and standard calibration curve

The sunscreen formulation with different formulation of *C. asiatica* extract was analysed for the anti-oxidant activity DPPH assay, where the percent radical scavenging activity of the DPPH was determined. From Figure 5, it can be seen that the scavenging activity increases as the concentration of extract increases. Comparing with standard ascorbic acid, none of the percentage scavenging assay value that suppress the value of ascorbic acid. For the formulated sunscreen, the scavenging activity increases a bit in F10 and rises to 38% at F20, which shows that the it has the highest antioxidant activity than the rest of the formulation. The sunscreens were studied along with antiradical agents, which indicates that the formulated sunscreens and the *C. asiatica* extract contains potential of substances to serve as hydrogen providers or free-radical scavengers.

Analysis of antimicrobial activity

The Antimicrobial activities was testing based on Kirby-Bauer's disc diffusion method and microbial inhibition as shown Table 4 and Table 5. The testing was performed on two type of bacteria which are *E. coli* and *S. epidermidis* which is negative and positive gram bacteria respectively.

Table 5: Diameter of the zone of inhibition of sunscreen for antimicrobial testing against *S. Epidermidis*

Formulation	Zone of Inhibition (mm)
F0	8
F5	9
F10	14
F15	11
F20	16

* F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract

Table 6: Diameter of the zone of inhibition of sunscreen for antimicrobial testing against *E. Coli*

Formulation	Zone of Inhibition (mm)
F0	10
F5	9
F10	11
F15	14
F20	15

* F0: sunscreen without *C. asiatica* extract, F5: sunscreen incorporated with 5% concentration of *C. asiatica* extract, F10: sunscreen incorporated with 10% concentration of *C. asiatica* extract, F15: sunscreen incorporated with 15% concentration of *C. asiatica* extract, F20: sunscreen incorporated with 20% concentration of *C. asiatica* extract

Antimicrobial susceptibility testing based on Kirby-Bauer's disc diffusion method and the zone of inhibition can be seen in Table 5 and Table 6. The formulations showed a higher sensitivity to gram-positive bacteria (*S. Epidermidis*) compared to gram-negative bacteria (*E. coli*) as the inhibition zone can be seen clearly. This is indicated by the value of the inhibition zone in *S. Epidermidis* is greater than that of *E. coli*. There is a high inhibition at F20 is known to have higher activity compared to that of lower concentration.

The result varied as the sunscreen contains extract at different concentrations. The antibacterial effects of extracts mainly were identified due to their flavonoid components [14]. Flavonoid increased the ability to form complexes with bacterial cell walls and the permeability of the bacterial cell surface to the extract. This is likely that the formulation has a much higher molecular weight for the lower concentrations. Molecules with high molecular weight cannot spread easily on bacterial cell walls compared to low molecular weight compounds [16].

4. Conclusion

In conclusion, this research focused on the study of sunscreen formulation incorporated with *Centella asiatica* extract and the evaluation of the sunscreen lotion. *C. asiatica* extract was incorporated in the formulation of sunscreen lotion with the aimed to produce the best formulation of a new organic sunscreen with good physicochemical, antimicrobial and antioxidant properties. The results show that the pH values of *C. asiatica*-containing sunscreen lotions in an average physiological level of 5.0-8.0 and stabilizes at F10 to F20 concentration at neutral pH value which is around 7.25 ± 0.012 value. The sunscreen also shows that it did not have any phase changes after placed in room temperature for 1 week. F20 has the highest SPF value among the others which is 22.21 by calculating absorbency using Mansur equation. The sunscreens were then tested on their blocking ability by comparing the survivability of microbe under the sunlight against the survivability of microbe protected with sunscreen under the sunlight. It is also observed that there is an antimicrobial inhibition on *S. epidermis* and *E. coli* growth that indicates there are antimicrobial activities. F20 shows biggest inhibition zone which

16mm when tested against *S. epidermidis* and 15mm when tested against *E. coli.* Based on this research, it can be concluded that sunscreen F20 incorporated with *C. asiatica* has the better properties among the other formulation and can be studied further.

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