CHAPTER 13
PHYSICOCHEMICAL PROPERTIES OF SOAP BAR FORMULATED USING NATURAL COLOURANT AND FRAGRANCE FROM *CLITORIA TERNATEA* AND *CITRUS HYSTRIX*

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INTRODUCTION

Soap is one of essential personal care products used for cleaning and washing in our daily tasks. It is a cleansing agent made from alkaline bases and fatty acids through a process referred as saponification. Thus, it consists of sodium or potassium salt of such acids. Natural soap or soap using natural ingredients is a simple variation of soap where natural bioactive ingredients are added to give more variety of biological activities in the final products. Plant extracts are a rich source of bioactive ingredients that can be developed into antibacterial, anti- inflammatory and antioxidant agents. Other than that, many plant extracts have long been used as remedy for common diseases, fragrances, colourant, as well as cosmetics. Soaps incorporated with these active extracts have higher market value for their additional beneficial activities on top of the common cleansing and washing properties.

Clitoria ternatea (*C. ternatea*) or the butterfly pea can be found in Asia, Africa, America and Australia. The plants are usually grown in garden as ornamental plants for its vivid deep blue flowers and as revegetation species to improve soil quality, and the roots, seed and leaves of the plants are used as brain tonic to promote higher memory in the Ayurvedic medicinal system (Gomez & Kalamani,

2003; Mukherjee et al., 2008). In the Southeast Asian countries, the flowers of *C. ternatea* are commonly being used for culinary purposes as food colourant to dessert, herbal drink, and traditional dish of "nasi kerabu". The blue pigments from the flowers extracts of *C. ternatea* has high stability in food preparation, thus, commonly used as food colourant. The major active compounds found in *C. ternatea* flowers anthocyanin of ternatin derivatives and flavonol glycosides (Kazuma et al., 2003b, 2003a; Nair et al., 2015). These polyphenols are known to have beneficial antioxidant and antimicrobial properties that can be useful for the bio properties of soaps (Leong et al., 2017).

Meanwhile, *Citrus hystrix* (*C. hystrix*) or commonly known as kaffir lime, is native to the tropical Southeast Asian and Southern China. This citrus plant is also valued for culinary purposes to add flavour and aroma in some traditional local soup dishes and being used to eliminate meat and fish odour during food preparation. The major compound of *Citrus hystrix* leaves is the L-citronellal that gives the mix scent of lemongrass and citrus aroma (Sato et al., 1990; Ratseewo et al., 2016).

The usage of natural ingredients in product formulation, such as soaps and cosmetics, is becoming a trend and is preferred by consumers. Although the usage of *C. ternatea* flowers and *C. hystrix* leaves in food preparation is very common, their application as colourant and fragrance in soaps is limited due to stability issues of the extracts in the product formulation (Habib et al., 2016). Addition of the plant extracts may also modify the physicochemical properties of the soaps. Thus, this study investigates the physicochemical properties of soap bars formulated using *C. ternatea* flowers and C. *hystrix* leaves extracts as natural colourant and fragrance.

MATERIALS AND METHODS

Chemicals and Reagents

C. ternatea flowers, *C. hystrix* leaves, 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, USA), ethanol, distilled water, sodium hydroxide, Mueller-Hinton agar (Oxoid, England), nutrient broth, chloramphenicol (Calbiochem, Germany), *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, 6 mm paper disc and Whatman no. 1 filter papers, sodium hydroxide and palm oil. All chemicals used were of analytical grade.

Sample Acquisition

The dried flowers of *C. ternatea* was purchased from an herbal store in Selangor and *C. hystrix* leaves were collected near Batu Pahat, Johor. Both samples were authenticated and stored at dry place until further use.

Sample Preparation

C. ternatea Flowers

The dried flowers of *C. ternatea* were ground into powder and was weighed (100 g). Later, the dried powdered was soaked in ethanol (500 ml) for 3 days at room temperature. The extracts were then filtered through No.1 Whatman filter paper. An ethanolic crude extract was collected and then was vaporised to dryness using rotary evaporator alanine aminotransferase, billirubin and glutathione with histopathological analysis. The dried flowers of C. ternatea were ground into powder and was weighed (100 g). Later, the dried powdered was soaked in ethanol (500 ml) for 3 days at room temperature. The extracts were then filtered through No.1 Whatman filter paper. An ethanolic

crude extract was collected and then was vaporised to dryness using rotary evaporator (Nithianantham et al., 2013).

C. hystrix Leaves

The leaves of *C. hystrix* were cleaned, washed and cut into small pieces. The leaves were weighed (50 g) and placed in a round bottom flask filled with distilled water (500 ml). The flask was connected to a condenser and *C. hystrix* leaves were distilled with water–steam for 4 hours. The experiment was stopped as the *C. hystrix* leaves were dried and essential oil was collected. The collected oil was dispensed in a bottle and stored at 4 ºC until further analysis (Nanasombat and Lohasupthawee, 2005).

Determination of Antioxidant Activity

Both *A. galanga* and *K. galang*a extracts were prepared at various concentrations ranging from 0-500 µg/ml. Later, 2.5 ml of samples at various concentrations were added to 4 ml of 0.004% DPPH in ethanol and incubated for 30 min at room temperature in the dark. Finally, absorbance readings at 517 nm were recorded and the percentage of scavenging activity were calculated.

$$\text{Percentage of scavenging activity (\%)} = \left(\frac{A_o - A_e}{A_o}\right) \times 100;$$

Where, Ao = absorbance without extract and Ae = absorbance with extract.

Determination of Antimicrobial Activity

The antimicrobial activity was analyzed using disc diffusion assay (Nanasombat & Lohasupthawee, 2005). Mueller-Hinton agar plates were streaked with a cotton swab containing 100 µl of respective

bacteria broth culture (*Staphylococcus aureus*, *Escherichia coli*). Later, a paper disc (6 mm diameter) was soaked with 10 µl of ethanolic extract, and other discs were in 10 µl of ethanol (negative control) and chloramphenicol (positive control). All soaked discs were dried in laminar flow (30 min) and were placed aseptically apart from each other on the agar with flamed forceps. The disc was pressed gently to ensure it completely touch to the surface of the agar. The plates were labelled, inverted and incubated for 24 h at 37 ºC. The diameter of the inhibition zones in mm against the test bacteria was measured.

Soap Bar Formulation

Soap formulations were calculated using a lye calculator software with 5% superfatting. Four formula were prepared F1=no extract added (control), F2=*C. ternatea* extract added, F3=*C. hystrix* extract added and F4=both extracts added, as shown in Table 13.1.

Table 13.1: Formulation of Soap Bars

Chemicals/ Ingredients	**F1**	**F2**	**F3**	**F4**
Lye (NaOH) (g)	6.84	6.84	6.84	6.84
Palm oil (g)	50.00	50.00	50.00	50.00
Distilled water (g)	15.30	15.30	15.30	15.30
***C. ternatea* (g)**	-	3.61	-	1.805
***C. hystrix* (g)**	-	-	3.61	1.805

Physicochemical Properties of Soap Bar

The physicochemical properties of the soap bars were evaluated using previous methods with some modifications (Ahmad Warra & Wawata, 2012; Afsar & Khanam, 2016). The soap bars were analysed for pH, colour and foamability using methods as described in Table 13.2.

Table 13.2: Physicochemical Properties of Soap Bar Formulation

Analysis of soap bar	Methods
pH	pH was determined by using a digital pH meter. Soap shavings (10 g) was weighed and soaked in distilled water before taking the pH reading.
Colour	Colour was determined by a calorimeter analyzer, which was touched to the surface of the soap bar to obtained L*, a* and b* value.
Foamability	0.5 g of soap and 20 ml of distilled water were added in 10 ml measuring cylinder. The mixture was shaken for about 3 minutes and the foam height was recorded.

Colour Differences

The total colour differences were calculated using the following formula to indicate the colour difference between sample and the commercial soap.

$$\text{Total colour difference, } \Delta E* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where, L* indicates lightness, a* measures the red or green coordinate and b* measures the yellow or blue coordinate.

Statistical Analysis

All statistical analysis was performed using GraphPad Prism 7.0. One-way ANOVA with Tukey multiple comparison was performed and $p<0.05$ is considered as significant.

RESULTS AND DISCUSSION

Antioxidant Activity

Both *C. ternatea* and *C. hystrix* extracts can scavenge DPPH free radical, as shown by the IC_{50} value of the extracts in Table 13.3 The result shows the IC_{50} values of *C. hystrix* extract (98.1 µg/ml) is lower than *C. ternatea* extract (4053.8 µg/ml), suggesting higher potency. However, both extracts have higher IC_{50} values than ascorbic acid (28.1 µg/ml).

Antimicrobial Activity

Antimicrobial activity of *C. ternatea* flowers and *C. hystrix* leaves extracts were conducted against *S. aureus* and *E. coli* respectively. The inhibition zones of the extracts from both *C. ternatea* and C. *hystrix* at two different concentrations against two bacteria species was shown in Table 13.3. Kamilla et al., (2009) has shown that the inhibition zones of *C. ternatea* flowers extract against *S. aureus* and *E. coli* were 13 mm and 13.3 mm respectively. However, in this study, the extracts from *C. ternatea* flowers showed inhibition zones against the Gram-positive bacterium of 6.33 mm and 7.00 mm at 500 and 1000 µg/ml respectively. Meanwhile, no inhibition zone was observed against Gram-negative bacterium.

The extract from *C. hystrix* leaves has shown antimicrobial activity against both of *Staphylococcus aureus* and *Escherichia coli*. The results (Table 13.4) indicated that *C. hystrix* leaves had shown inhibition against *S. aureus* at 500 and 1000 µg/ml with 7.00- and 7.33-mm zone of inhibitions and *E. coli* at 1000 µg/ml (6.67 mm). Similar results were found by Sidek and Abdullah (2018), where *C. hystrix* leaves showed antimicrobial activity against the negative bacterium, *E. coli*.

Table 13.3: IC_{50} of Ascorbic Acid, *Clitoria ternatea* Flowers and *Citrus hystrix*

Samples	IC_{50} (µg/ml)
Ascorbic acid	28.1
C. ternatea	4053.8
C. hystrix	98.1

Table 13.4: Antimicrobial Activity of *C. ternatea* and *C. hystrix*

Samples	Type of Bacteria	Concentration (µg/ml)		Positive control (Chloramphenicol)	Negative control (Ethanol)
		500	1000		
***Clitoria ternatea* flowers**	EC	-	-	+	-
	SA	+	+	+	-
***Citrus hystrix* leaves**	EC	-	+	+	-
	SA	+	+	+	-

** EC = Escherichia coli and SA = Staphylococcus aureus. (+) showed inhibition zone and (–) showed no inhibition zone*

Physicochemical Properties of Soap Bar Analysis

Colour Analysis

In this study, the soap bar colour was assessesd using a colourimeter analyzer. The colourimeter analyser showed the value of L*, a* and b*. The colour of soap bar formulation was compared with the commercial soap. The data for colour analysis was as shown in Table 13.5. F1 was less light and less yellow in colour than the commercial soap. The total colour difference between F1 and standard soap (Dettol soap) was 11.34. F2 was less light, greener and less yellow than the standard soap, Dettol with 23.93 total colour difference. F3 was lighter and less yellow than the Dettol soap with total colour difference of

11.68 between the soaps. F4 was less light, greener and less yellow than the Dettol soap, with total colour difference 14.54 between the two soaps.

Table 13.5: Colour Difference between Soap Bar Formulation and Commercial Soap

Sample code		Soap Bar (Sample)	Commercial soap (Standard)	Colour different	Colour Description
F1	**L***	66.73	64.25	ΔL* = +2.48	More light
	a*	4.67	-4.47	Δa* = +9.41	-
	b*	16.07	21.90	Δb* = -5.83	Less yellow
				ΔE* = 11.34	
F2	**L***	41.83	64.25	ΔL* = -22.42	Less light
	a*	3.77	-4.47	Δa* = +8.24	More green
	b*	20.44	21.90	Δb* = -1.46	Less yellow
				ΔE* = 23.93	
F3	**L***	66.47	64.25	ΔL* = +2.22	More light
	a*	4.77	-4.47	Δa* = +9.24	-
	b*	15.11	21.90	Δb* = -6.79	Less yellow
				ΔE* = 11.68	
F4	**L***	52.00	64.25	ΔL* = -12.25	Less light
	a*	3.30	-4.47	Δa* = +7.77	More green
	b*	20.90	21.90	Δb* = -1.00	Less yellow
				ΔE* = 14.54	

**F1=No extract soap, F2=C. ternatea soap, F3=C. hystrix soap, F4=C. ternatea +C. hystrix soap*

pH Analysis

The pH value obtained from soap bars formulation were 10.33 to 11.46 as shown in Table 13.6: Table shows pH reading between soap bar formulation and commercial soap. Data were mean±SD of triplicates. Different alphabets indicate significant difference at $p<0.05$., where all soap bars formulations have significantly higher pH ($p<0.05$) than

the standard soap (9.87). Meanwhile, F2 (added with *C. ternatea*) and F3 (added with *C. hystrix*) has no significant pH difference but significantly lower than F1 (no extract), suggesting that addition of extracts in the soap formulation may lower the pH value of the soap. Similar trend was observed when F4 (*C. ternatea* + *C. hystrix*) has lower pH than F1, F2 and F3. All the four samples of soap bar formulation had higher pH than the commercial soap. According to a study by Habib et al., (2016) higher pH value may indicate incomplete hydrolysis of saponification due to shorter curing time. Thus, longer curing time may lower the pH value of the soaps. Overall, all soap bars formulations showed alkaline pH value.

Table 13.6: pH Reading between Soap Bar Formulation and Commercial Soap

Sample	pH of Soap Bar Formulation/Standard soap
F1	11.46 ± 0.05^{a}
F2	11.16 ± 0.05^{b}
F3	11.13 ± 0.05^{b}
F4	10.33 ± 0.05^{c}
Standard (Dettol)	9.87 ± 0.05^{d}

**Data were mean±SD of triplicates. Different alphabets indicate significant difference at $p<0.05$.*

Foamability Analysis

The foamability of all four soap bar formulations was measured based on foam height as shown in Table 13.7. Standard soap (Dettol) has the highest foamability. In this experiment, foamability comparison was focused to F1 (no extract) since standard soap may include other foaming agents. Addition of extracts F2 (addition of *C. ternatea*) and F3 (addition of *C. hystrix*) significantly lowers the foaming ability

of the soaps (p<0.005). However, a mix of both extracts, as in F4, significantly enhances the individual foaming ability of the extracts in soap formulations (F2, F3), with p<0.05.

Table 13.7: Foamability Test between Soap Bar Formulation and Commercial Soap.

Sample	Height foam of Soap Bar Formulation/ Standard soap (ml)
F1	6.83±0.24b, d
F2	6.33±0.24c
F3	6.00±0.00c
F4	7.00±0.00b
Standard (Dettol)	9.00±0.00a

** Data were mean ±SD of triplicates*
*** Different alphabets indicate significant difference at p<0.05*

CONCLUSION

As a conclusion, the physicochemical properties of the pH and foamability of the soaps was modified with the addition of the extracts (*C. ternatea* and *C. hystrix*) in the soap bar. Addition of *C. ternatea* extract (F2) affect the colour to become greener, whilst addition of *C. hystrix* (F3) has no effect as compared to the standard soap (F1). All formulated soaps are within the alkaline range but addition of *C. ternatea* and *C. hystrix* can affect the formulation by lowering the pH. Although both extracts cause less foam, a mix of both extracts increases the foamability of the soap. Other than that, the antioxidant and antimicrobial activities exhibited by the extracts may further add value to the formulated soaps. The data from this study may help soap manufacturers to innovate soap from *C. ternatea* and *C. hystrix* extracts.

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