The Determination of Antioxidant and Antimicrobial Analysis of Gaharu Essential Oil and Gaharu Leaves in Soap Formulations.

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***Abstract*— In this study, the new utilization of gaharu main components which is gaharu essential oil and gaharu leaves was conducted by integration into the soap formulation. Six different soap formulation were prepared using hot process soap making. The analysis in the soap performance was conducted on the soap stability such as appearance attribute, pH and foaming ability. Furthermore, antioxidant and antibacterial abilities was conducted using 2,2-DiphenylPicrylHydrazyl (DPPH) assays and Kirby Bauer Disk Diffusion assay were co respectively. These studies show that the soap samples added with gaharu essential oil provides a good soap attribute such as appearance, odor and good antioxidant activity while the soap samples added with gaharu leaves yield no added attribute such as good odor and pleasant appearance but possess a good antioxidant free radical scavenging activity of 71.83 percent radical scavenging activity. The antimicrobial analysis shows highest inhibition for coconut soap added with gaharu essential oil of 14 mm, while there is no inhibition of olive soap samples even with addition of gaharu essential oil.**

Keywords*— *Agarwood Liquid Soap, Antioxidant Activity, Antibacterial Activity.

In this modern era, hygienic attribute is crucial in maintaining the overall health aspects due to currently increasing in skin illnesses such as the Hand, Foot and Mouth Disease (HFMD) that can contribute to fatality. Therefore, the used of skin care product was highly suggested and has becomes the common routine in every household in both developed and developing country.

Basically, soap is cleansing agent that become the basic building block of skin care product and exist in variety forms such as body soap, hand soap and facewash. The main basic components of soap making is fats or oil and alkali. Fats and oils are triglycerides of fatty acid that is originated from animals or plants sources or a combination between those two. Moreover, the degree of saturation does differentiate between fats and oils by which the fats is the saturated ones and oils the unsaturated counterparts. Furthermore, sodium hydroxide (NaOH) and potassium hydroxide (KOH) are alkali that is widely used in production of hard (bar soap) and soft (liquid soap) soap respectively. Next, batch and continuous process govern the production of traditional and modern soap respectively. Modern soap making can be produced through several routes which is the direct neutral fats saponification where the natural source of fat is being saponified with alkali. Next, through fatty acids saponification, where the natural fatty acid first undergoes hydrolytic splitting to separate glycerol with fatty acids before the pure fatty acid been saponified with alkali. Then, through the saponification of fatty acid methyl esters [1]. Figure 1 illustrate the chemical reaction that occur between triglycerides of fatty acid with three molecules of alkali to produce the fatty acid metal salt and glycerin that made up the overall composition of soap.

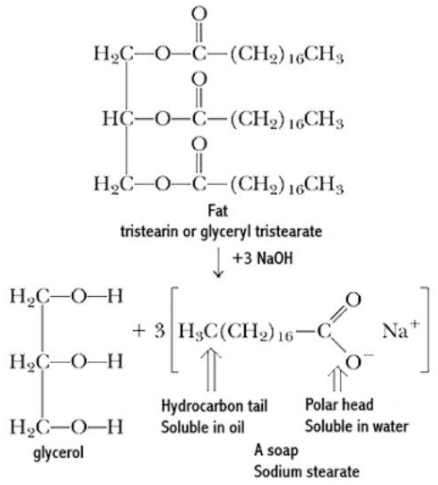

Figure 1.0: Molecular Interaction in Saponification Process [2]

Currently, major production of skin care product was derived from synthetic sources while there are minimal natural products available. Most modern skin-cleansing product is integrated with complex chemicals, containing not only surfactants, but also skin-conditioning agents and a variety of other ingredients to add color and scent, to improve stability, to aid in processing and manufacturing, or to modify the product's in- use performance [12]. Surfactant is said to having a problem regarding the aquatic lives since the small amount of some type of the surfactant such as cationic surfactant even at low concentration is toxic [14]. Furthermore, the FDA ban on triclosan the antimicrobial agents used in various skin care products does raise the consumer awareness on chemically active ingredient. In addition, current trends in consumer preference have shown an increase in demand for the use of natural ingredients in personal skin care and cosmetics products [13]. Moreover, natural antioxidants in the form of plant extracts are typically added as additives (1–8% of final soap composition) to suppress the oxidation of polyunsaturated fatty acids in natural herbal soaps [17]. Gaharu plant is believed to possess the antioxidant in its oleoresin extract as well as in the leaves.

Gaharu is one of the names besides agarwood, heartwood, eaglewood and aloeswood that is famously known in the Malaysia and Indonesia region. Gaharu is economical plant that well known for its unique properties of the extracted oleoresin and commonly being derived into certain product such as incense, traditional medicines and perfume. Formation of agarwood occurs in the trunk and roots of trees that have been infected by parasites. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding the infection and this process is called Tylosis [2]. This pathological condition of gaharu yield the formation of oleoresin at the trunk of the trees is due to its defense mechanism against the infections namely parasitic ascomycetous mould and phaeoacremonium parasitica, a dermatiaceous (dark walled) fungus [3]. Aquilaria Malaccensis exist in several species that usually differentiated based on sizes of the tree, uses and geographical location. Gaharu Leaves is believed to exhibit an antioxidant ability that brings an interest among the researches. Past study conducted by Moosa (2016) states that the extract of the gaharu plant yield a positive result when it was subjected under free radical scavenging activities using 2,2-Diphenyl-1-Picyrlhydrazyl (DPPH) assay [5].

Biochemical reaction occur in human body may be altered towards the negatives impact such as cancer, resulted from the increase exposure to the environment and diet that increase the risk to oxidative damage of cells and DNA. This is due to generation of chemical components in our body that changes to the reactive species namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS). The way to fight this cellular damage is through the use of antioxidant. Antioxidant are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage and can be classified into multiple ways based on its activity [10]. Basically, antioxidant was classified into enzymatic and non-enzymatic where enzymatic ones work by breaking down and removing free radicals while non-enzymatic counterpart interrupt the radical chain reaction. Non-enzymatic antioxidant exists abundantly in plant-based species that possess complex constituents of chemical such as vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione [10].

This research focus was to determine the antioxidant and antimicrobial activities of gaharu components that comprises of gaharu essential oil and gaharu leaves to be incorporated in the soap. Since past study on the gaharu chemical composition is believed to contain useful cytotoxic chemicals that provides a good antioxidant characteristic, and to explore the new antibacterial properties that gaharu components can brings into the soap characteristics to explore the new areas to utilize this valuable plant that growth widely in south east Asia region.

METHODOLOGY

A. Materials

The matured gaharu leaves were collected at Jalan Kebun Shah Alam. The leaves were rinse using flowing tap water and wipe to remove the water and dirt on the surface of the leaves. Next, the leaves were dried by using vacuum far infrared dryer to ensure the optimum condition and preservation of the important phytochemicals needed inside the leaves. The drying process was operated at 50°C and 0.6 bar for 120 minutes [9]. Furthermore, after the leaves has cool to a room temperature then it is ready for grinding process. The leaves were cut into small pieces and been inserted into dry mill to obtain the dry powder.

Coconut Oil (Medella) and Olive Oil (Naturel) were purchased at the local store while gaharu essential oil was purchased at the BF1 Malaysia.

B. Soap Making (Hot Process)

Six different formulation were formulated using coconut and olive as two different soap bases. This is to determine the stability of gaharu essential oil and leaves in both saturated and unsaturated types of oils. Three samples from coconut oil and three from olive oil were incorporated with gaharu essential oil and gaharu leaves powder and the remaining were leave as blank soap from both types of oils. Hot process method was used in producing the soap bases because the saponification can be accelerated to completion. The soap was produced at a basis of 200 grams of oils. The calculations of alkali required were determined based on the saponification value of each oil type using the soapcalc.com websites to prove the manual calculation conducted as the illustration in figure 2.

For coconut liquid soap, the 200 grams of coconut oil was weighed and place in a mixer bowl. The 40% of alkali concentration was used to prepare the alkali solution. Pour 51.40 gram of potassium hydroxide (KOH) solid into the 77.10 gram of distilled water. The alkali solution was allowed to cool down to 30^0C. After that, the alkali solution was added to the coconut oil. The mixtures were mixed using the mixer until it reaches the slurry thick phase (trace phase). The trace was heated for the duration of 3 to 4 hours and in the interval of 30 minutes the trace was stirred using spatula. Finally, the translucent soap gel was formed and was diluted with distilled water with the ratio of 1:3 respectively. After that, the mixture was allowed to dissolve completely for a duration of 24 hours. Next, the liquid soap was separated in 3 bottles. First sample was leave blank while second and third sample was incorporated with 5 percent of gaharu essential oil and gaharu leaves powder respectively. The samples added with gaharu essential oil and leaves were heated at around 60°C. All the samples were labelled as in abbreviations. The steps were repeated for olive oil soap.

Abbreviations:

CO – Coconut Oil Soap
COG – Coconut Oil Soap with 5% Gaharu Essential Oil
COGL – Coconut Oil Soap with 5% Gaharu Leaves Powder
OO – Olive Oil Soap
OOG – Olive Oil Soap with 5% Gaharu Essential Oil
OOGL – Olive Oil Soap with 5% Gaharu Leaves Powder

SoapCalc © Recipe Name: New INCI Names Print Recipe

Total oil weight	200 g	Sat : Unsat Ratio	89 : 11
Water as percent of oil weight	38.55 %	Iodine	10
Super Fat/Discount	0 %	INS	258
Lye Concentration	**40.0000 %**	Fragrance Ratio	0
Water : Lye Ratio	1.5000:1	Fragrance Weight	0.00 g

	Pounds	Ounces	Grams
Water	0.170	2.72	77.10
Lye - **KOH**	0.113	1.81	51.40
Oils	0.441	7.05	200.00
Fragrance	0.000	0.00	0.00
Soap weight before CP cure or HP cook	0.724	11.59	328.50

#	√	Oil/Fat	%	Pounds	Ounces	Grams
1		Coconut Oil, 76 deg	100.00	0.441	7.05	200.00
		Totals	100.00	0.441	7.05	200.00

Soap Bar Quality	Range	Your Recipe		
Hardness	29 - 54	79	Lauric	48
Cleansing	12 - 22	67	Myristic	19
Conditioning	44 - 69	10	Palmitic	9
Bubbly	14 - 46	67	Stearic	3
Creamy	16 - 48	12	Ricinoleic	0
Iodine	41 - 70	10	Oleic	8
			Linoleic	2

Figure 2.0: The soap component calculation using SoapCalc.com

C. pH Analysis

The pH of the soap samples was tested using pH meter (Mettler Toledo). The pH electrode was dipped into the test sample and result was taken and recorded.

D. Foaming Abilities

10 ml of liquid soap sample was dissolved in 10 ml distilled water and then was shaken vigorously in measuring cylinder for 10 seconds. The height of the suspended foam was recorded.

E. Antioxidant Testing

The soap samples were subjected to DPPH (Sigma Aldrich) Free Radical Scavenging Assay. DPPH was chosen due to the highest sensitivity towards free radical scavenging activity. The result from inhibitory plots indicated that the concentration-dependent DPPH anti radical assay presented a more sensitive and efficient method than the ABTS assay. (Paper 5) The soap samples were diluted up until the concentration of 7500 µg/ml by diluting 3.75 grams of soap with 500 ml water. Next, 1 ml of each soap samples was reacted with l ml of 0.2 mM DPPH in 95% methanol. The solution was then incubated in dark at room temperature for 30 min and the absorbance (A) was measured at 517 nm using a spectrophotometer. All samples were performed in triplicates. The scavenging activity was calculated as a percentage of DPPH discoloration relative to a negative control using the following equation [6]

Free-radical scavenging activity (%) = [((A (blank) - A (extract))/ A (blank)] x 100 ——— Equation 1

Blank: DPPH + Methanol (buffer solution)
Extract: Soap Samples

F. Anti-Bacterial Testing

The antibacterial testing was studied using the gram negatives Escherichia Coli (E-Coli) and gram-positive Lactobacillus, by adopting the Kirby Bauer Disk Diffusion Method which is an effective test to evaluate bacterial sensitivity towards potential antimicrobial compounds. Sizes or inhibition zones is the indicator of sensitivity of the tested bacterium towards the compounds. The Nutrient Agar was used as the medium for Kirby Bauer Disk Diffusion Assay. The nutrient agar was prepared by dissolving 5 grams nutrient powder in 250 liters distilled water. The solution was sterilized in an autoclave at 120 ^{0}C for 15 minutes. The solution allowed to cool down and was poured into the petri dish. One petri dish is approximated to has 20 ml volumes and were allowed to solidify. The E-Coli bacteria was inoculated using sterile cotton swab onto the agar surface. The 6mm filter paper disc was soaked with the soap samples and was located at the agar medium aseptically. The media was incubated for 24 hours to allow the colony to grow. The zone of inhibition was measured using ruler and recorded.

II. RESULTS AND DISCUSSION

A. Appearance Attributes

The appearance of the soap depends on the types of oils being used. The coconut oil soap known for its clear white color while olive oil soap possesses yellowish color that contribute in the light yellowish of the olive oil soap samples. Apart from that, when the gaharu leaves powder was incorporated the darker green color was visible but this depends on the percentage of the gaharu leaves powder added and it can vary from dark green to yellowish in color. This may due to the chlorophyll chemical in the leaves that make up for the green in color. Table 1 below represents the variation of color for the soap samples.

Table 1: Appearance of the soap samples

No	Soap Samples	Color
1	CO	Clear
2	COG	Light Yellowish
3	COL	Dark Green
4	OO	Yellow
5	OOG	Yellow
6	OOL	Dark Green

B. The Soap Stability Testing

I. pH Testing:

pH of the samples was tested using both the digital pH meter and pH paper. From table 1, the olive soap shows the highest pH of 10.20 while olive soap with addition of 5% gaharu essential oil and olive oil with gaharu leaves powder yield pH of 9.48 and 8.65 respectively. Apart from that, the samples from coconut soap shows a pH of 9.5 while coconut soap incorporate with gaharu essential oil and leaves display a pH value of 9.10 and 8.22 respectively. The samples from both type of soap samples exhibits the same trends, where the trends are decreasing with the addition of gaharu essential oil and gaharu leaves powder with addition of gaharu essential oil does lowering the pH a little bit and addition of gaharu leaves powder is the major reduction of pH. Furthermore, the pH of the soap samples also been tested using pH paper and shows the value of 8-11 in the range of body soap [3]. This pH value is important because if the soap is not fully saponified it will possess a caustic effect to the skin. Therefore, pH is the main parameter to be analyze for soap. Table 2 and figure 3 below represents the data for the all tested soap samples with different formulations.

Table 2: The pH of the soap samples by pH meter

Samples	CO	COG	COGL	OO	OOG	OOGL
pH	9.50	9.10	8.22	10.20	9.48	8.65

II. Foaming Abilities:

Foam is created when the surface tension of water (attraction of surface molecules toward the center, which gives a drop of water its round shape) is reduced and air is mixed in, causing bubble formulation. In this soap formulation the result was tabulate in table 3. The foaming abilities for the coconut soap, the addition of gaharu essential oil samples yield the highest foaming volume of 60-mm. The shorter chain saturated fatty acids in coconut or palm oil increase the lathering profile of the final soap products due to enhanced solubility in water [17].

Lather is a characteristic that many consumers find aesthetically pleasing and often is viewed as an indicator of a skin cleanser cleaning ability [12].

Table 3: The Foaming Abilities of Soap Samples

Samples	CO	COG	COGL	OO	OOG	OOGL
Height of Soap Foams (mm)	45	60	35	41	48	30

C. *Antioxidant Analysis*

The order of highest percentage of radical scavenging vary in decreasing order from the addition of gaharu leaves, without addition of gaharu and with addition of gaharu essential oil. The highest discoloration was on the subject added with gaharu leaves with 71.83 percent for coconut soap base and 66.09 percent for olive oil soap base. Soap samples obtained from the 5 percent addition of leaves extract and 5 percent addition of gaharu essential oil while the soap samples added only with leaves extract vary around 71 to 74 percent of DPPH free radical scavenging. In comparison, the antioxidant of the leaves extract is lower than leaves powder in water (Gaharu Tea). Maharani et al, (2016) states that, antioxidant activity for all types of Borneo Agarwood leaves extract with hot water extraction (400x dilution) showed a higher percentage than its ethanol. It is mainly due to the fact that boiling water could completely activate the degradative enzymes as against the ethanol solvent [8]. The IC_{50} value of ascorbic acid is 0.219 mg/ml at the concentration of 1mg/ml. If this value is scale to 7 times, since our soap concentration is 7.5 mg/ml, the value of relative towards this study is 1.533 mg/ml. The ranges of IC_{50} for the soap samples is around 5 to 7 which means the antioxidant power of the soap is 5 to 7 times weaker than the ascorbic acid which has the highest antioxidant ability. This may due to liquid soap is already diluted with water that reduces the real concentration even more for the antioxidant assays.

Blank absorbance of DPPH Solution: 2.300

Table 4: The absorbance of the soap samples with different formulations.

Soap Sample	Absorbance at 517 nm	Percentage of Free Radical Scavenging Activity (%)	IC_{50} (mg/ml)
CO	0.895±0.005	61.09	6.14
COG	1.155±0.004	49.78	7.53
COL	0.648±0.016	71.83	5.22
OO	1.008±0.003	56.17	6.68
OOG	1.190±0.001	48.26	7.77
OOL	0.780±0.004	66.09	5.67
Gaharu leaves in water	0.332±0.003	85.57	4.38

D. *Antibacterial Analysis*

The soap samples were subjected under Kirby-Bauer disk diffusion methods where gram-positive and gram-negative bacteria were used namely lactobacillus and e-coli. Soap samples with addition of 5 percent of gaharu essential oil yield zone of inhibition of 9 mm on e-coli while 14 mm on lactobacillus while the samples of coconut soap without gaharu essential oil shows low inhibition of 8 and 7 mm for e-coli and lactobacillus respectively. Apart from that, olive oil soap both blank and addition of gaharu show no inhibition toward the bacteria. The inhibition of the COG and OOG can be seen higher towards the lactobacillus species over the E-Coli this may be due to the facts that the gram negatives of e-coli does possess the additional outer layer of lipopolysaccharides that encapsulate the peptidoglycan that exist as single cell wall for the gram-positive species. The thin cell wall makes the bacterial species vulnerable to the bioactive compounds that present in many plant species, ultimately gaharu, since the oleoresin formation is believed as the defend mechanism against the physical wounding and fungal infection.

Table 9: The zone of inhibition of the soap samples.

Soap Samples	Zone of Inhibition (mm)	
	E-Coli	Lactobacillus
CO	8	7
COG	9	14
COGL	8	8
OO	S	S
OOG	7	10
OOGL	S	S

III. Conclusion

In conclusion, the liquid soap samples prepare using the coconut oil and olive oil incorporated with gaharu essential oil and gaharu leaves show the good stability, enhance foaming, good appearance, good antioxidant activity and minimal microbial susceptibility.

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