Physicochemical Properties of Noni Fruit, Yam Root, Rose Petal, and Betel Leaf Transparent Soap and Their Antimicrobial Activities

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Abstract. Natural soap is prepared without an artificial surfactant, and with the addition of functional ingredients such as a natural substance, essential oil, or plant extract. The present study was to perform transparent soap formulation enriched with noni (Morinda citrifolia L.) fruit, yam (Pachyrizus erosus) root, rose (Rosa damascena) petal, and betel (Piper betle L.) leaf extract and investigate its antibacterial activity. These herbal transparent soaps were made by hot process method. Physicochemical characteristics tested were moisture content, pH, total fatty matter, free fatty acid content, and chloride content. Antimicrobial activities of herbal transparent soaps were investigated by agar well diffusion method. The result showed that transparency was maintained when the moisture content was greater than 17% but not exceed about 21%. pH value of herbal transparent soap ranged from 9.22 to 9.47, while the free fatty acid content ranged from 0.12% to 2.15%. Yam root transparent soap showed the highest total fatty matter (45.12%). While the lowest total fatty matter was shown by the betel leaf transparent soap (23.20%). The chloride content of noni fruit, yam root, rose petal, and betel leaf transparent soap was 14.38%, 14.17%, 21.81%, and 16.52%, respectively. Noni fruit, yam root, betel leaf transparent soaps had stronger antimicrobial inhibitory than control soap, while the rose petal transparent soap had comparable antimicrobial inhibitory with control. All herbal transparent soap and control at the concentration level of 10% and 20% exhibited antibacterial activity against all microorganism tested (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans) to a certain degree.

Keywords: transparent soap, Morinda citrifolia L., Pachyrizus erosus, Rosa damascena, Piper betle L., antibacterial activity

1. Introduction

Nowadays, the harmful effects of synthetic surfactants, the environmental friendliness, and environmental sustainability of large – scale commercial soap making in the factory are being emphasized. On the contrary, natural soap is prepared without an artificial surfactant, with the addition of functional ingredients such as a natural substance, essential oil, or plant extract, and typically run in small scale by home/small industry. Based on the making method, natural soap may be generally divided into a melt – pour soap, a hot process soap, and a cold process soap. The hot process soap is
also named a transparent or translucent soap. This soap is made by mixing caustic soda with oil at a high temperature. To make the hot soap, the elements should be heated in a water bath or directly heated for 40 to 50 minutes, and thereafter, the heated elements should be matured in normal temperature for 2 weeks. The soap has good detergency or cleansing power, good moisturizing effects, long-lasting fragrance, and less of an irritant.

Aucubin, L-asperuloside, alizarin, and scopoletin in the Noni (Morinda citrifolia L.) fruit are proven, antibacterial agents. These compounds have been shown to fight against infectious bacteria strains such as Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella, and Shigella. [1, 2]. Extracts from the ripe noni fruit exhibited moderate antibacterial properties against Pseudomonas aeruginosa, M. pyrogenes and Escherichia coli [3]. Locher reported that selected plants including noni have a history of use in Polynesian traditional medicine for the treatments of infectious disease [4]. Essential oil, absolute and hydrosol of Rosa damascena has wide spectrum antimicrobial activities. The in vitro antibacterial activities of essential oil from Rosa damascena were exhibited inhibitory and bactericidal activities against gram-positive Staphylococcus aureus (ATCC 25923), gram-negative Escherichia coli (ATCC 25922), gram-negative Pseudomonas aeruginosa (ATCC 27853), and yeast Candida albicans (ATCC 14053) [5, 6]. Antibacterial effect of major components of rose oil (citronellol, geraniol, and nerol) was reported [7]. Antibacterial properties of rose absolute could be attributed to its high phenyl ethyl alcohol content [8]. Piper betle L. has been demonstrated in vitro to have significant antimicrobial potential against a broad spectrum of microorganisms including bacteria and fungi and has been further described as an effective alternative to reduce biofilm formation in membrane bioreactors caused by both Pseudomonas aeruginosa and Candida albicans [9, 10, 11, 12]. The antimicrobial potential is most likely due to hydroxychavicol, stearic acid, and palmitic acid. The antimicrobial activity explains the use of betel leaf as an oral care agent in Asian countries [13]. Betel leaf has also displayed the greatest potential value, against both Gram-negative and Gram-positive multidrug-resistant bacteria [14]. Further, some herbal ingredients have been added to the soaps to add its functionality. The present study was to perform transparent soap formulation enriched with this functional herbal extract and investigate its antibacterial activity.

2. Materials and Methods

2.1. Preparation of herbal extract

Selected herbal used in this study were noni (Morinda citrifolia L.) fruit, yam (Pachyrizus erosus) root, rose (Rosa damascena) petal, and betel (Piper betle L.) leaf. The ripe noni fruit and yam root extract were pureed and squeezed to collect the juice. Each of fresh juice was evaporated to dryness under reduced pressure at 45 °C. While rose petal and betel leaf extract prepared by collecting the hydrolat (hydrosol, aromatic water) from the steam distillation process. Hydrolat obtained during distillation of aromatic plants. Hydrolat can be collected since a small hydrophilic fraction, oxygenated, odor imparting, water-soluble oil constituents escape into the distillation/condensate water stream.

2.2. The making process of transparent soap

Stearic acid (8.5%), citric acid (0.3%), virgin coconut oil (32%), ricini oil (1%), and alcohol 96% (14%) were mixed while heated until the blend solution was clear at a temperature range between about 50 to 60 ℃. Neutralized the batch by slowly adding an aqueous solution of sodium hydroxide while ensuring the temperature does not exceed about 85 ℃. After aqueous sodium hydroxide has been added, the mixture was kept between about 80 to 85 ℃ for a while to ensure the saponification reaction was complete. Added aqueous mixture of sucrose and propylene glycol to the batch and mixed vigorously until solution was clear. While maintained gently agitation, cooled the batch to a temperature about 60 ℃ and slowly added herbal extract (8%) and mixed until a homogenous mixture was achieved. The composite was then poured into molds and allowed to solidify for 24 hours. The cooled transparent soap removed from the molds and allowed in normal temperature for 2 weeks for aging process.
2.3. Physicochemical properties of soap

Determination of physicochemical properties of soap includes pH, moisture content, total fat matter, free fatty acid content, and chloride content. Standard procedures as described by AOCS [15], were used for the analysis of physicochemical properties with slight modifications. pH of 10 % solution of transparent soap was determined by means of a digital pH – meter (Eutech PC 700). Moisture content was measured by moisture analyzer digital (AND MX – 50). Method for measurement of total fat matter, free fatty acids, and chloride content was gravimetric, volumetric, and argentometric methods, successively.

2.4. Antimicrobial activity

The bacterial strains used to assess the antimicrobial properties of herbal transparent soap included one Gram-positive (Staphylococcus aureus) and two Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and one fungus (Candida albicans). The investigated microbial strains were obtained from the Department of Agricultural Microbiology, Universitas Gadjah Mada, Indonesia. The antimicrobial activities of herbal transparent soaps were evaluated by agar well diffusion [16, 17] using Nutrient Agar medium for the bacterial assay and Potato Dextrose Agar medium for the fungal assay. The microorganism was activated by inoculating a loopful of the bacterial strain in the Nutrient Broth (10 mL) and incubated at temperature 37 °C for 24 hours, while the fungal strain in the Potato Dextrose Broth (10 mL) and incubated at temperature 30 °C. Then 100 µl of bacterial inoculum (inoculum size was 10⁸ cells/ml as per McFarland standard) was inoculated into the Nutrient Agar media (20 mL) and fungal inoculum into the Potato Dextrose Agar media (20 mL). After proper homogenization, it was poured into Petri dishes. Wells were made in the seeded plates with the help of a cup-borer (7 mm). 25 µL of test solution and control solution (Lifebuoy® transparent soap) at a level concentration of 1%, 5%, 10%, 20% were introduced into the well. Bacterial plates were incubated at 37 °C for 24 hours and fungal plates were incubated at 30 °C for 48 hours. The experiment was performed 3 times under strict aseptic conditions.

3. Results and Discussion

The soap bar of this study has gloss-like clarity. The fatty acids – stearic acid contribute to lathering and washing properties of the soaps [18]. Coconut oil is a source of lauric acid (12 carbons) which can be made into sodium laurate. This soap is very soluble and will lather easily. Fatty acids with only 10 or fewer carbons are not used in soaps because they irritate the skin and have objectionable odors [19]. The amount of fatty acid to be neutralized with a polyol and or polyol blend. The presence of non-volatile polyols enhances the clarity of the product and prevents shrinkage of the bar during storage and use. The sodium hydroxide was used in bar soap making as alkalis that react with glycerides to provides further neutralizing activity for production of optimum transparency. These are naturally different from soaps made from divalent metals such as calcium, magnesium, iron or aluminum which are not soluble in water [19]. Since the neutralization of the fatty acid is an exothermic reaction, addition of sodium hydroxide must be controlled consequently the temperature will not exceed 85 °C.

3.1. Physicochemical evaluation

The physicochemical characteristic of soap depends on several factors which include the strength and purity of alkali, the kind of oil used and completeness of saponification. Physicochemical characteristics include moisture content, pH, total fatty matter, free fatty acid, and percentage chloride [20]. Good quality soap for cleansing purpose is one that strikes a balance in all the mentioned physicochemical parameters. The results obtained from the analysis of different physicochemical parameters of the different soaps are shown in Table 1.

Water is an important ingredient because the hardness and clarity of the finished soap are strongly dependent on its total moisture content. There are several sources of water in this formulation such as the caustic soda solution and the water generated during the neutralization reaction. The addition of free water to the bar formulation will also influence the final product. Generally, water addition of less than 5% total (not formed in situ or introduced by other ingredients) will usually result in a bar that is too hard and tends to form crystals with associated loss of clarity. Free water addition more than about
15% will usually result in a bar that is too soft. The present study showed that transparency was maintained when the moisture content was greater than 17% but not exceed about 21%. Higher moisture content in soap would lead to a reaction of excess water with unsaponified fat to give free fatty acid and glycerol in a process called hydrolysis of soap on storage.

**Table 1. Physicochemical properties of transparent herbal soap**

| Parameters          | Noni fruit | Yam root | Rose petal | Betel leaf | Control |
|---------------------|------------|----------|------------|------------|---------|
| Moisture content    | 17.69% dw  | 18.94% dw| 20.98% dw  | 18.91% dw  | 20.23% dw|
| pH                  | 9.31       | 9.22     | 9.47       | 9.65       | 9.18    |
| Total fatty matter  | 37.75%     | 45.12%   | 39.17%     | 23.20%     | 36.80%  |
| Free fatty acid     | 0.65%      | 2.15%    | 0.77%      | 0.12%      | 0.08%   |
| Chloride content    | 14.38%     | 14.17%   | 21.81%     | 16.52%     | 59.12%  |

A correct pH range and the use of an adjusting agent are critical for achieving transparent soap bars from starting formulations. It has been discovered that adjusting the pH within a range of 9 to 9.5 will result in the desired end products. Obtaining a pH outside the preferred range will opacify the product. The high pH indicates a high percentage amount of unspecified and unsaponifiable matter due to incomplete alkaline hydrolysis. pH and free fatty acid content are critical balance to obtain an acceptable transparent product. Free fatty acid content and pH correlated with objective observations for transparency in the end products. Adding citric acid increased the free fatty acid content but decreased the pH. pH value of herbal transparent soap ranged from 9.22 to 9.65, while the free fatty acid content ranged from 0.12% to 2.15%. The transparency of end products was sustained when the pH did not drop under 9.1 and the free fatty acid content did not exceed 4.0%.

The total fatty matter is one of the most important characteristics describing the quality of soap and it is always specified in commercial soap. It is defined as the total amount of fatty matter, mostly fatty acids. Total fatty matter is usually associated with hardness and quality of soaps [21]. The soap with the higher total fatty matter gives more lather, lasts longer and, more importantly, cleans your skin better and more gently. Yam root transparent soap showed the highest total fatty matter (45.12%). While the lowest total fatty matter was shown by the betel leaf transparent soap (23.20%). These differences in the total fatty matter are possibly due to the imperfection of saponification process. The lower total fatty matter value may be caused by the presence of unreacted NaOH in the mixture [22].

The determination of percentage chloride levels in soap is important by means of excess quantity causes soaps to crack [23]. The chloride content of noni fruit, yam root, rose petal, and betel leaf transparent soap was 14.38%, 14.17%, 21.81%, and 16.52%, respectively. The cause of high chloride content in the soap is due to the use of chlorinated water to dissolve NaOH pellets [23].

3.2. Antimicrobial activity

The bacteria and fungus studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents. Antimicrobial activities of herbal transparent soap were determined by measuring the diameter of the zone of inhibition and the mean values are presented (Table 2).

All herbal transparent soap and control at a concentration level of 10% and 20% exhibited antibacterial activity against all microorganism tested to a certain degree. However, all herbal transparent soap and control were inactive against *P. aeruginosa* and *C. albicans* at a concentration level of 1% and 5%. Noni fruit transparent soap at a concentration level of 20% exhibited the strongest antimicrobial inhibitory against *S. aureus* dan *P. aeruginosa*. Yam root transparent soap at a concentration level of 10% and 20% exhibited the strongest antimicrobial inhibitory against *C. albicans*, though the inhibition zone was relatively small (9.5 – 11.97 mm). Betel leaf transparent soap at all concentration level exhibited the strongest antimicrobial inhibitory against *E. coli*. 
Table 2. Antimicrobial activities of transparent herbal soaps

| Soap sample   | Concentration (%) | S. aureus | E. coli | P. aeruginosa | C. albicans |
|---------------|-------------------|-----------|---------|---------------|-------------|
| Control       | 1                 | 15.91     | 13.02   | 0             | 0           |
|               | 5                 | 19.38     | 17.49   | 0             | 0           |
|               | 10                | 20.93     | 18.9    | 9.9           | 9.49        |
|               | 20                | 22.64     | 20.27   | 11.5          | 11.34       |
| Noni fruit    | 1                 | 15.68     | 15.87   | 0             | 0           |
|               | 5                 | 21.07     | 18.47   | 0             | 0           |
|               | 10                | 23.72     | 19.65   | 10.77         | 10.09       |
|               | 20                | 25.22     | 22.08   | 12.81         | 11.62       |
| Yam root      | 1                 | 16.95     | 14      | 0             | 0           |
|               | 5                 | 19.33     | 18.04   | 0             | 0           |
|               | 10                | 23.26     | 19.38   | 10.8          | 9.5         |
|               | 20                | 24.83     | 20.74   | 12.33         | 11.97       |
| Rose petal    | 1                 | 14.58     | 13.29   | 0             | 0           |
|               | 5                 | 19.47     | 17.7    | 0             | 0           |
|               | 10                | 21.6      | 18.58   | 10.53         | 9.11        |
|               | 20                | 22.5      | 20.55   | 10.88         | 10.38       |
| Betel leaf    | 1                 | 14.87     | 15.57   | 0             | 0           |
|               | 5                 | 19.3      | 20.3    | 0             | 0           |
|               | 10                | 22.22     | 20.87   | 10.98         | 9.31        |
|               | 20                | 24.56     | 23.9    | 12.75         | 10.96       |

S. aureus was the most susceptible and C. albicans was the most resistant among all the microorganism strains investigated in the present work. All herbal transparent soaps were more active against the S. aureus than the Gram-negative bacteria and even C. albicans. This comes to an agreement with previous reports that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria [24, 25].

4. Conclusion
The present investigation demonstrates that noni (Morinda citrifolia L.) fruit, yam (Pachyrizus erosus) root, rose (Rosa damascena) petal, and betel (Piper betle L.) can be formulated into a transparent soap to add its functionality. Physicochemical properties and antibacterial activity of herbal transparent soaps were comparable to control soap.

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6. References
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