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Characterisation and Antibacterial Activity of Green Tea Extract-Enriched Solid Goat's Milk Soap

Uswatun Chasanah*, Dian Ermawati, Dwi Putri Utami, Angela Nora Hayati
Department of Pharmacy, University of Muhammadiyah Malang, Malang, East Java, Indonesia

ABSTRACT

Solid goat's milk soap is organic and beneficial for the health and appearance of the skin. One of the components in green tea known to display antibacterial activities is epigallocatechin gallate (ECGC). In this study, the effects of solid goat's milk soap containing 1%, 2%, and 4% green tea extract on the growth of *Propionibacterium acnes, Staphylococcus aureus,* and *Staphylococcus epidermidis* were examined. The soap was characterized for its organoleptic, pH, free fatty acid, alkalinity, water content, foam stability, and hardness. In addition, an in vitro antimicrobial test was performed utilising the well-diffusion method. The findings revealed that all the soap formulas matched the SNI 3533-2016 standards for water content, free fatty acid content, and free alkali content, and that they also had similar pH, foam stability, and hardness. The solid goat's milk soap showed antibacterial activities against *Propionibacterium acnes, Staphylococcus aureus*, and *Staphylococcus epidermidis*, but the effect of the green tea extract on the soap did not increase these.

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**corresponding author*
Email: uswatun@umm.ac.id

INTRODUCTION

Goat's milk has been used in bath soap for a long time (Pandya & Ghodke, 2007; Ribeiro & Ribeiro, 2010). The content of the milk, such as fat, protein, minerals and vitamins (Park et al., 2007), are helpful for skin health. One of the ingredients of goat's milk is a natural beta hydroxy acid which has an antiaging effect (Moghimipour, 2012), promotes skin brightness and smoothness, and is not scaly. The proteins in goat's milk are nutrients that moisturise and coat the surface of the skin, making it smoother and more supple (Gorouhi & Maibach, 2009).

Natural ingredients can be added as antibacterial agents to improve the efficacy of solid goat's milk soap. Previously, such soap had not been enriched with green tea extract, so we chose it as an enriching ingredient. Catechin is the content of green tea with antibacterial properties. There are four main types of catechin, namely epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallic (EGCG), with ECGC as the highest constituent (Senanayake, 2013). It has been reported that green tea extract can inhibit the growth of bacteria found in the skin that causes acne vulgaris, *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes*. The minimum inhibitory concentration (MIC) of green tea extract against *Propionibacterium acnes, Staphylococcus aureus*, and *Staphylococcus epidermidis* is less than 0.400 mg/mL (Li et al., 2015; Sharma et al., 2012; Yoon et al., 2013).

This research aimed to determine the characteristics of solid goat's milk soap containing green tea extract and to reveal its antibacterial activity against *Propionibacterium acnes, Staphylococcus aureus*, and S*taphylococcus epidermidis*.

METHODS

Materials

The cell culture bacteria used in the study were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, and *Propionibacterium acnes* ATCC 11828, obtained from CV Wiyasa Mandiri, Singosari, Indonesia, and identified macroscopically using nutrient agar media and microscopically using a staining test from the Laboratory of Microbiology, Medical Faculty, University of Muhammadiyah Malang, Indonesia. The ingredients for the antibacterial study were nutrient agar (Merck), sterile water, and McFarland standards 10^8 CFU/ml, prepared from a 1% solution of anhydrous barium chloride (Merck) and a 1% solution of sulfuric acid (Merck). The soap ingredients were fresh goat's milk obtained from Batu City, olive oil (Textron, Spain), palm oil (Sunco, Indonesia), coconut oil (Aroma Nusantara, Indonesia), sodium hydroxide flakes (Hisea, China), titanium dioxide (Tianjin, China), fragrant green tea oil (Parfex, France), green tea extract (PT Bali

Table 1. Formulation of solid goat's milk soap enriched with green tea extract

Ingredients	Composition (gram)			
	Control (0%)	FI (1%)	FII (2%)	FIII (4%)
Fresh goat's milk	30	30	30	30
Green tea extract	-	1.5	3.0	6.0
Aqua distillate	7.5	6.0	4.5	1.5
NaOH	14.4	14.4	14.4	14.4
Coconut oil	30	30	30	30
Palm oil	30	30	30	30
Olive oil	35	35	35	35
Titanium dioxide	0.6	0.6	0.6	0.6
Green tea fragrance oil	2.5	2.5	2.5	2.5
Weigh of soap	**150**	**150**	**150**	**150**

Ekstrak Utama, Indonesia), ethanol 96%, potassium hydroxide (Merck), a 1.0% solution of phenolphthalein (Merck), and distilled water.

Soap Formulation

Solid goat's milk soap containing green tea extract at concentrations of 1% (w/w), 2% (w/w), and 4% (w/w) was then made, while the formulation functioning as a negative control was similar soap but without green tea extract. The soap was formulated according to the calculation results to ensure that the characteristics of the soap produced met soap quality requirements. The formula for the soap is shown in Table 1.

The solid goat's milk soap enriched with green tea extract was prepared via cold-process saponification. Sodium hydroxide was added directly to frozen milk. Titanium dioxide was then dispersed in olive oil, coconut oil and palm oil. The sodium hydroxide solution in the goat's milk was poured into a mixture of oils to form an intimate mix, blended until thin or medium trace. Green tea extract and fragrance were added prior to pouring the saponification mixture into moulds. The soap was allowed to harden by air-drying for 24 hours to obtain the soap bar. The bar soap was then left for 3 weeks to cure. Each soap formula was produced in triplicate.

Characteristic Analysis

Organoleptic

Organoleptic observation was made visually by noting the soap's shape, colour, smell and homogeneity. A homogeneity test was conducted by placing 0.1 g of soap on the surface of an object glass, which was then covered with another object glass. If there was no lump, it could be stated that the soap was homogeneous.

Determination of pH

pH testing was performed using a calibrated pH meter (Metler Toledo). 10 g of soap was dissolved in 20 ml of distilled water, then a pH meter was placed in the solution, allowing the pH value to be obtained.

Determination of total fatty matter/total alkali content

Determination of total fatty matter/total alkali content was made according to National Standardization Agency (2016) guidelines. Four grams of crushed solid soap was placed in 200 mL of neutral ethanol, and the mixture was heated in a water bath until it dissolved, and was then filtered. The filtrate was then obtained when heated. When the filtrate was nearly boiling, 0.5 mL of phenolphthalein solution was added. If the solution was acidic (indicated by colourless of the phenolphthalein indicator), the contents were titrated against standard KOH 1 N solution until a stable pink colour appeared. If the solution was alkaline (indicated by red colour of phenolphthalein indicator), the contents were titrated against a standard solution of HCl 1 N until red colour disappeared.

The moisture content was tested based on the weight loss method after being heated at a temperature of 105°C ± 2°C. The test was conducted by inserting 5 ± 0.01 g of sample into a petri dish heated at 105°C ± 2°C for 1 hour and then cooled in a desiccator to room temperature. The sample was heated again for 1 hour until a constant weight was obtained.

Foam stability

A foam stability test was performed by weighing 1.0 g of the soap bar and then dissolving it in 10 mL of water. If needed, the mixture could be heated to speed up solubility at 70°C. 3 mL of the solution was placed in a

scale test tube, and 3 mL of water was added and then shaken for 20 seconds. The foam formed was observed, and then its height was recorded, and after being left for 15 minutes, it was measured again.

Hardness of the soap

A test of the hardness of the soap was conducted on a 1 cm x 1 cm x 1 cm piece of soap, which was placed on a manual tablet hardness tester. The force applied until the soap was crushed was recorded.

Antibacterial Activity

Sample preparations

Five grams of soap from each formula, including soap obtained from the market containing triclosan 2% (with brand name X) as a positive control, was dissolved in 5 ml of distilled water.

Antibacterial activity test

An antibacterial activity test of the solid goat's milk soap enriched with green tea extract was conducted using the agar-well diffusion method (Balouiri et al., 2016). First, five holes with a diameter of 6 mm were made in NAP media that had previously been inoculated with *Propionibacterium acne, Staphylococcus epidermidis,* or *Staphylococcus aureus*. Each well contained 20 µl of liquid from solid goat's milk soap without green tea extract as a negative control, solid antibacterial soap sold on the market as a positive control, and solid goat's milk soap containing green tea extract at 1%, 2%, and 4% concentrations as test samples. The presence of antibacterial activity from the sample could be seen from the large inhibition zone formed around the well after incubation at 37°C for 18–24 hours.

Statistical Analysis

Data on the physical and chemical characteristics, including pH, water content, free fatty acid, total free alkali, hardness and foam stability, as well as zone inhibition from the antibacterial test results, were analyzed using one-way analysis of variance at 0.05 degrees of confidence using the Statistical Package for the Social Sciences (SPPS) v16.0 program.

RESULTS AND DISCUSSION

Results

All the formulas showed a white to brownish colour, depending on the level of green tea extract concentration, with an aromatic smell of green tea, as described in Figure 1. The results of the homogenity test are shown in Figure 2.

The organoleptic properties of all the soap formulas were as expected. Titanium dioxide made the appearance of the soap opaque and attractive, while the fragrant oil of green tea strengthened its aroma. The characteristics included organoleptic, pH, moisture content, free fatty acid, hardness, and foam stability, as shown in Table 2.

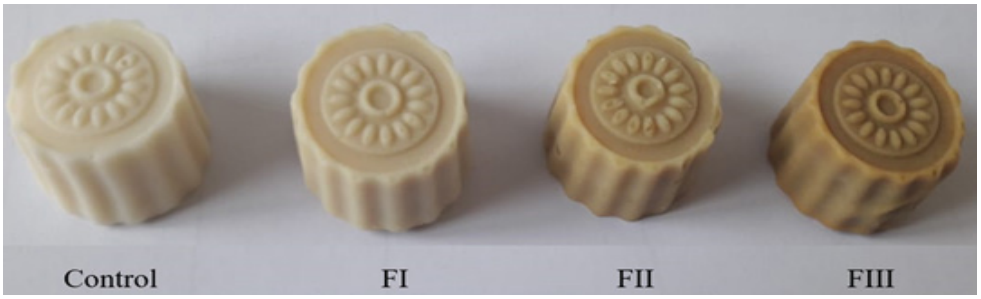

Figure 1. Solid goat's milk soap without green tea extract (control) and with green tea extract at concentrations of 1% (FI), 2% (FII), and 4% (FIII).

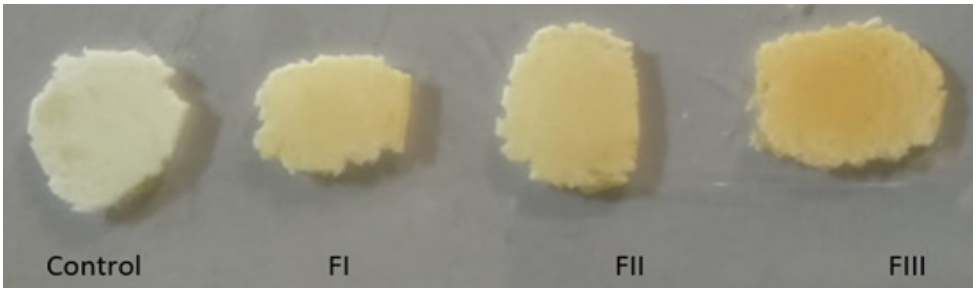

Figure 2. Results of the homogeneity test of solid goat's milk soap without green tea extract (control) and with green tea extract at concentrations of 1% (FI), 2% (FII), and 4% (FIII).

Table 2. Characteristic of goat's milk bar soap enriched with green tea extract (average ± SD, n = 3)

Sample	Colour	Homogeneity	pH	% Water content	% Free fatty acid	% Foaming stability	Hardness (kg)
Goat's milk soap without green tea extract	White	Homogenous	10.64 ± 0.41	0.54 ± 0.11	2.23 ± 0.56	75.98 ± 4.06	3.0 ± 0.0
Goat's milk soap + 1% green tea extract	Brownish white	Homogenous	10.74 ± 0.34	0.61 ± 0.12	2.37 ± 0.63	78.13 ± 4.70	3.0 ± 0.0
Goat's milk soap + 2% green tea extract	Light brown	Homogenous	10.86 ± 0.24	0.51 ± 0.13	2.41 ± 0.57	78.91 ± 2.04	3.0 ± 0.0
Goat's milk soap + 4% green tea extract	Brown	Homogenous	10.81 ± 0.37	0.64 ± 0.07	2.52 ± 0.29	77.80 ± 3.18	3.0 ± 0.0

The purpose of adding green tea extract to the goat's milk solid soap formula was to increase its antibacterial activity. An X brand antibacterial soap containing triclosan 2% was used as a positive control. The results of the examination of the antibacterial activity are listed in Table 3.

Discussion

The critical chemical component of the surface structure of bacteria as cell wall peptidoglycans, teichoic acids, lipoteichoic acids, and the bacterial plasma membrane is composed primarily of protein and phospholipid (Salton & Kim, 1996). Soap molecules have hydrophilic heads and hydrophobic tails. When immersed in water, they are transformed into little bubbles called micelles, with their hydrophobic tails pointed inward. While the soap molecule tails want to avoid water, they attract oils and fats, including the bilayer membranes of bacteria, breaking them apart and destroying them (Clark, 2020). The solid goat's milk soap enriched with green tea extract inhibited the growth of *Staphylococcus aureus, Staphylococcus epidermidis*, and *Propionibacterium acnes*, as shown in Table 3. In *Staphylococcus aureus,* zone inhibition caused by soap with green tea extracts of 1%, 2% and 4% was not significantly different ($p = 0.729$), being 1.63 ± 0.25 cm, 1.53 ± 0.12 cm, and 1.53 ± 0.12 cm respectively. In *Staphylococcus epidermidis*, zone inhibitions caused by soap with green tea extract of 1%, 2%, and 4% were 0.90 ± 0.00 cm, 0.87 ± 0.06 cm, and 0.97 ± 0.12 cm respectively ($p = 0.453$). Similar results were also observed with *Propionibacterium acnes,* with the inhibition zones produced by soap with green tea extracts at 1%, 2%, and 4% also not being significantly different ($p = 0.679$), at 1.23 ± 0.12 cm, 1.17 ± 0.17 cm and 1.30 ± 0.14 cm respectively. These results show that the activity against the *Staphylococcus aureus* and *Propionibacterium acnes* bacteria is the same and stronger than the antibacterial activity against *Staphylococcus epidermidis*. When compared with goat's milk soap without green tea extract, it was shown that the zone inhibition produced by soap containing the extract was the same. It can be said that adding green tea extract to goat's milk soap does not increase its antibacterial activity. Such activity of green tea extract is due to its ECGC content (Widyaningrum et al., 2015; Yoon et al., 2013). When making goat's milk soap, a saponification reaction produces a high pH (9–11). At the same time, ECGC is stable under acidic conditions (Pękal & Pyrzynska, 2015), so it is unable to increase the antibacterial activity of the soap. It is known that the MIC of green tea extract is 0.400 mg/mL (Li et al., 2015), so such extract added to solid goat's milk soap to improve antibacterial activity of the soap. The presence of antibacterial activity in the sample can be seen from the large inhibition zone formed around the well after being incubated at 37ºC for 18–24 hours.

pH

After pH testing of all the soap formulas, it was found that the pH value of goat's milk soap without green tea

Table 3. Antibacterial activity of goat's milk bar soap enriched with green tea extract (average ± SD, n = 3)

	Inhibition zone (cm)		
	Staphylococcus aureus	***Staphylococcus epidermidis***	***Propionibacterium acnes***
Goat's milk soap without green tea extract (negative control)	1.47 ± 0.12	1.07 ± 0.15	1.20 ± 0.14
Goat's milk soap + green tea extract 1%	1.63 ± 0.25	0.90 ± 0.00	1.23 ± 0.17
Goat's milk soap + green tea extract 2%	1.53 ± 0.12	0.87 ± 0.06	1.17 ± 0.17
Goat's milk soap + green tea extract 4%	1.53 ± 0.12	0.97 ± 0.12	1.30 ± 0.14
Antibacterial soap "X" (positive control)	2.47 ± 0.12	1.93 ± 0.06	2.17 ± 0.21

extract was 10.64 ± 0.41, while those of soaps containing 1%, 2%, and 4% green tea extracts were 10.74 ± 0.34, 10.86 ± 0.24, and 10.81 ± 0.37. respectively. The pH of all the soap formulas was shown to be the same ($p = 0.880$). Therefore, we conclude that the pH of all the formulas were similar at 10.64–10.81, which is in the pH range of typical soap on the market (Tarun et al., 2014).

Propionibacterium acnes grew much better in pH 6.0–7.0 than in a more acidic or alkaline milieu. *Staphylococcus epidermidis* resembled *Staphylococcus aureus*, showing no significant difference at pH 5.5 and 7.0 (Korting et al., 1992). The study results showed a pH of the solid goat's milk soap in the range of 10.64–10.81. Therefore, even though the soap does not contain green tea extract, it can still inhibit the growth of bacteria. Moreover, some antibacterial soaps have the effect of drying the skin. Compared with existing antibacterial soaps, the advantage of solid soap made from goat's milk, which contains lipids, vitamins, and minerals, is that it can soften and brighten the skin more. Therefore, solid soap from goat's milk can be a better alternative for use as an antibacterial soap, especially in an anti-acne context.

Water content

The water content of all the goat's milk soap formulas was very similar; that of the soap without green tea extract was 0.54% ± 0.11%, while that of soap containing green tea extract at 1%, 2%, and 4% was 0.51% ± 0.13%, 0.61% ± 0.12%, and 0.64% ± 0.07% respectively. The water content of all the soap formulas can be said to be the same ($p = 0.454$). The requirements stated in SNI 3533-2016 about bar soap are that the water content is a maximum of 15%. In our formulas it was in the range of 0.51%–0.64%. Therefore, it can be concluded that the water content was low. As seen in Table 1, the formula for the goat's milk soap used a low amount of water. The curing period of 1 month may also result in evaporating the water content in the soap.

Free fatty acid/free alkali

The use of NaOH in the preparation of soap must be at the right concentration. If the amount is too high, the presence of free alkali with amounts exceeding the provisions will cause skin irritation and dry skin (Hernani et al., 2014). The tolerable limit of alkalinity levels is less than 0.1% (National Standardization Agency, 2016). The bar soap without and with green tea extract at levels of 1%, 2%, and 4% showed no colour change in the sample after adding a 1% phenolphthalein indicator, which indicated that none of the formulas contained free alkali. Therefore, they all met the requirements.

Furthermore, from the analysis of free fatty acids, the soap without green tea extract contained 2.23% ± 0.56%, while the soap with green tea extract at 1%, 2%, and 4% contained 2.37% ± 0.63%, 2.41% ± 0.57%, and 2.52% ± 0.29%, respectively, so all the formulas were very similar ($p = 0.930$). Compared with the requirement that the content of free fatty acids in solid soap should be less than 2.5% (National Standardization Agency, 2016), the content in FI and FII met the requirement. This high level of free fatty acids is because goat's milk contains up to 3.8% fat, and not all these fats underwent saponification reactions.

Foam stability

Soap that dissolves in water causes a decrease in surface tension, meaning that foam is formed. Soft and stable foam is the main feature of a soap product. Foam stability was measured by comparing the foam height after 15 minutes of foam formation with the initial height. The foam stability test result on soap without green tea extract was 75.98% ± 4.06% and the results

on soap with the green tea extract of 1%, 2%, and 4% were 78.13% ± 4.70%, 78.91% ± 2.04%, and 77.80% ± 3.18% respectively. Therefore, all the formulas are also very similar in this respect ($p = 0.794$). The content of lauric and myristic acid in coconut oil produces a soft foam, while the palmitic acid in palm oil produces a stable foam. In all the formulas, the soap contained the same composition of coconut oil, palm oil, and olive oil. Consequently, all the soaps had the same foam stability.

Soap hardness

The content of lauric acid ($C_{12}H_{24}O_2$) in the coconut oil produces hard soap, as does the content of palmitic acid ($C_{16}H_{32}O_2$) found in oil palm. As the oil composition in all the formulas was the same, the results of the hardness test related to soap both with and without green tea extract did not differ, with all the formulas exhibiting a hardness of 3.0 kg ± 0.0 kg

CONCLUSION

We prepared solid goat's milk with green tea extract at levels of 1%, 2%, and 4%. The characteristics of all the formulas, which included pH, water content, free fatty acid as oleic acid, foam stability and hardness were similar. However, the addition of green tea extract did not increase the antibacterial activity. It is vital to develop an acidic liquid soap formula of green tea extract with non-irritant ingredients to obtain a soap with an optimum antibacterial effect.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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REFERENCES

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis,6*(2), 71–79.

Clark, C. (2020). *Getting back to chemistry basics : How simple soap saves lives*. https://news.emory.edu/stories/2020/03/esc_soap_saves_lives/campus.html

Gorouhi, F., & Maibach, H. I. (2009). Role of topical peptides in preventing or treating aged skin. *International Journal of Cosmetic Science, 31*(5),327-345.

Hernani, H., Bunasor, T. K., & Fitriati, F. (2016). Formula sabun transparan antijamur dengan bahan aktif ekstrak lengkuas (*Alpinia galanga* L. Swartz.) [Antifungal transparent soap formula with active ingredient galangal extract (*Alpinia galanga* L. Swartz.)]. *Buletin Penelitian Tanaman Rempah dan Obat, 21*(2), 192-205.

Korting, H. C., Lukacs, A., Vogt, N., Urban, J., Ehret, W., & Ruckdeschel, G. (1992). Influence of the pH-value on the growth of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Propionibacterium acnes* in continuous culture. *Zentralblatt fur Hygiene und Umweltmedizin = International Journal of Hygiene and Environmental Medicine*, *193*(1), 78–90.

Li, Z., Summanen, P. H., Downes, J., Corbett, K., Komoriya, T., Henning, S. M., Kim, J., & Finegold, S. M. (2015). Antimicrobial activity of pomegranate and green tea extract on *Propionibacterium Acnes, Propionibacterium granulosum, Staphylococcus aureus,* and *Staphylococcus Epidermidis*. *Journal of Drugs in Dermatology, 14*(6), 574–578.

Moghimipour, E. (2012). Hydroxy acids, the most widely used anti-aging agents. *Jundishapur Journal of Natural Pharmaceutical Products, 7*(1), 9–10.

National Standardization Agency. (2016). *Sabun Mandi Padat (SNI 3532:2016) [Solid Bath Soap (SNI 3532: 2016)].* Jakarta: National Standardization Agency.

Pandya, A. J., & Ghodke, K. M. (2007). Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research, 68*(1-2), 193-206.

Park, Y. W., Juárez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research, 68*(1-2), 88-113.

Pękal, A., & Pyrzynska, K. (2015). Effect of pH and metal ions on DPPH radical scavenging activity of tea. *International Journal of Food Sciences and Nutrition*, *66*(1), 58–62.

Ribeiro, A. C., & Ribeiro, S. D. A. (2010). Specialty products made from goat milk. *Small Ruminant Research, 89*(2-3), 225-233.

Salton, M. R. J., & Kim, K. S. (1996). Chapter 2. Structure. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston. https://www.ncbi.nlm.nih.gov/books/NBK8477/

Senanayake, S. N. (2013). Green tea extract: Chemistry, antioxidant properties and food applications - A review. *Journal of Functional Foods, 5*(4), 1529-1541.

Sharma, A., Gupta, S., Sarethy, I. P., Dang, S., & Gabrani, R. (2012). Green tea extract: possible mechanism and antibacterial activity on skin pathogens. *Food Chemistry*, *135*(2),672–675.

Tarun, J., Susan, J., Suria, J., Susan, V. J., & Criton, S. (2014). Evaluation of pH of bathing soaps and shampoos for skin and hair care. *Indian Journal of Dermatology, 59*(5), 442–444.

Widyaningrum, N., Fudholi, A., Sudarsono, & Setyowati, E. P. (2015). Stability of epigallocatechin gallate (EGCG) from green tea (*Camellia sinensis*) and its antibacterial activity against *Staphylococcus epidermidis* ATCC 35984 and *Propionibacterium acnes* ATCC 6919. *Asian Journal of Biological Sciences, 8*(2), 93-101.

Yoon, J. Y., Kwon, H. H., Min, S. U., Thiboutot, D. M., & Suh, D. H. (2013). Epigallocatechin-3-gallate improves acne in humans by modulating intracellular molecular targets and inhibiting *P. acnes*. *The Journal of Investigative Dermatology*; *133*(2), 429–440.