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Research Article

Formulation and Evaluation of a Turmeric Kombucha Facial Toner with Potential as an Anti-Acne Agent

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Abstract

Propionibacterium acnes is one of the gram-positive bacteria that causes acne. Many acne treatments use synthetic drugs that have side effects. One alternative for treatment is using fermented products such as kombucha. The potential of kombucha as an anti-acne can be increased by adding turmeric during the fermentation process. The product is Turmeric Kombucha, which will be applied to the skin. The cosmetic dosage form developed is Facial Toner. The research to be carried out aims to formulate and test the activity of turmeric kombucha facial toner preparations against *P.acnes* bacteria. The novelties of this research include increasing the anti-acne activity of Turmeric Kombucha and the addition of Turmeric Kombucha as an active ingredient in facial toner preparations that meet the evaluation requirements, activity tests, and stability tests. The stages of the method started with Turmeric Kombucha fermentation with variations in turmeric concentration and fermentation time. The fermentation results will be tested for antibacterial activity using the paper disc diffusion method. Turmeric Kombucha, which has the highest activity, is then used as an active substance in facial toner preparations, which will be evaluated and tested for stability. The results of the evaluation of facial toner preparations, all formulas meet the requirements for organoleptic, pH, and viscosity tests. The stability test results for facial toner preparations show that F3 is a stable formula at room temperature storage and extreme temperatures. Based on the results of the irritation test, kombucha turmeric facial toner did not irritate. The antibacterial test results showed that all formulas could inhibit P.acnes bacteria with F3 as the formula with the most effective inhibition diameter of 7.33 ± 0.57 mm, then F1 and F4 6.33 ± 0.57 mm, and F2 6 ± 0 mm. This study concludes that fermented turmeric kombucha can be formulated as an anti-acne facial toner.

Keywords: paper disc diffusion, *Propionibacterium acnes*, facial toner, Turmeric Kombucha

BACKGROUND

Acne is a chronic inflammation of the pilosebaceous follicles of the skin characterized by papules, pustules, nodules, open or closed comedones, and cysts. A study by Hasanah & Novian (2020) states that almost everyone experiences acne at the age of 14-17 years, with the highest incidence reaching 83-85% [1]. Bacteria that can cause acne are *P.acnes* and staphylococcus epidermis. Until now, many acne treatments use antibiotics or synthetic products. Treatment of acne using antibiotics can lead to resistance and cause unwanted side effects [2,3]. Therefore it is necessary to develop natural medicines such as kombucha.

Kombucha is a fermented product of black tea or green tea with the addition of a medium that utilizes a starter culture of lactic acid bacteria [4,5]. Kombucha drink has therapeutic potential such as antimicrobial, antioxidant, anticarcinogenic, gastric ulcer treatment, and high cholesterol [6,7]. The medicinal benefits of Kombucha can be enhanced by adding other natural components. One of them can ferment regular kombucha with the addition of turmeric rhizome (Curcuma longa). Natural phenolic compounds, like those found in turmeric rhizome, include essential oils and curcumin [8,9]. In addition, turmeric rhizomes can treat various skin problems, including dermatitis, psoriasis, and acne [10]. Application of use to facial skin can be made in cosmetic preparations, one of which is toner.

Facial toner is a product that is used as a second cleansing agent after a facial cleanser. However, toner is currently more widely used as a cosmetic or skincare product to balance the skin's pH, moisturize the skin, anti-acne, and disguise pores [11]. One of the benefits of a toner that is widely used is an anti-acne toner. Based on this background, research has been conducted to formulate and test the activity of turmeric kombucha facial toner preparations against *P.acnes* bacteria.

MATERIALS AND METHODS

This research consists of six stages, including:

Preparation of tools and materials

The tools and materials used for fermentation and activity tests were sterilized using the wet heat sterilization method [12]. Meanwhile, the instruments and materials for facial toner formulation and evaluation are not fixed.

Turmeric Kombucha Fermentation

The Turmeric Kombucha Fermentation Process uses a modified Kim & Adhikari (2020) method [13]. The modifications made included: the addition of turmeric rhizome on the 7th, 10th, and 14th incubation days; fermentation time plus 7 days

after the addition of turmeric rhizome; and variations in the concentration of the acquisition of turmeric rhizome (10%, 15%, and 20%). The fermentation results were evaluated by measuring the pH using a pH meter.

Fermented antibacterial activity test

The activity test begins with the preparation of MHA media [14], then the *P.acnes* bacteria are inoculated [10]. After that, a 1 McFarlan or 10^8 CFU/ml bacterial suspension was made [15]. Antibacterial activity testing used the paper disc diffusion method (Kirby-Bauer Methode) with three different treatments of incubation time (7 days, 10 days, and 14 days) and variations of turmeric rhizome (10%, 15%, and 20%). In addition, samples of kombucha, distilled water, and clindamycin were used as controls. All work processes are carried out aseptically. The measuring parameter used is the diameter of the inhibition zone. The result of the largest diameter of the inhibition zone is used as an active substance in the preparation of kombucha turmeric face toner [16].

Formulation and evaluation of Turmeric Kombucha facial toner preparations

The preparation of facial toner consists of 3 formulas with variations in the addition of an active substance (Turmeric Kombucha) between 10% - 20% and a control formula (without active substance) (Table 1). Preparations will be evaluated, including organoleptic tests; pH tests; hedonic tests of 15 panelists; and Stability tests using the cycling test method for six cycles [17].

Table 1 Formulation of turmeric kombucha facial toner [11]

Material	**F1** **% (b/b)**	**F2** **% (b/b)**	**F3** **% (b/b)**	**F4** **% (b/b)**	**Function**
Glycerin	2	2	2	2	Humectants
Propylene glycol	3	3	3	3	Humectants
DMDM Hydantoin	0.6	0.6	0.6	0.6	Preservative
TEA	0.1	0.1	0.1	0.1	pH regulator
Turmeric Kombucha	10	15	20	-	Active substance
Aquadest ad	100 ml	100 ml	100 ml	100 ml	Solvent

Antibacterial activity test for Turmeric Kombucha facial toner

Testing the antibacterial activity using the paper disc diffusion method (Kirby-Bauer Methode) for the three formulations with distilled water as a negative control and clindamycin as a positive control [10].

Data analysis

Each data collection will be repeated three times. The data obtained will be processed statistically, one of which is the one-way ANOVA test using JASP software version 0.16.0.0 (open source).

RESULTS AND DISCUSSION

Turmeric Kombucha Fermentation

The fermentation of kombucha turmeric is carried out in two stages. The first fermentation is the preparation of kombucha, which consists of tea and sugar with the addition of SCOBY. The second fermentation is the addition of turmeric to the kombucha. Kombucha fermentation was carried out with three treatments, namely 14 days, 10 days, and 7 days. Tea and granulated sugar are the best substrates for making kombucha, although green tea can also be used [18]. The addition of sugar in fermentation functions as a food ingredient because yeast uses sugar as a substrate to produce organic acids [19]. The longer the fermentation time, the more acidic the resulting pH will be.

(a) (b) (c) (d)

Figure 1: Fermentation of Turmeric Kombucha (a) 14 days kombucha fermentation (b) 10 days kombucha fermentation (c) 7 days kombucha fermentation (d) turmeric kombucha fermentation

The difference in fermentation time affects pH, cellulose thickness, biological activity, and taste. A fermentation time of 14 days produced the sourest taste, the thickest cellulose, and the lowest pH (Table 2). During the fermentation process, glucose is polymerized and has cellulose and hemicellulose, and this causes the more extended the fermentation time, the thicker the cellulose or nata that is formed [18]. Kombucha's sweet taste diminishes with longer fermentation time because SCOBY utilizes sugar as a substrate to produce organic acids.

The second fermentation is the addition of turmeric rhizomes to the fermented kombucha (Figure 1). The second fermentation was carried out for 7 days. Turmeric rhizomes are known to be alkaline, so adding turmeric to kombucha will increase the final pH. In addition to affecting the pH, the addition of turmeric rhizome affects the color and taste of kombucha to turn yellow and have a distinctive turmeric smell.

Table 2: Kombucha fermentation 14 days

Days to-	Temp	Results
0	26°C	The SCOBY starter is placed in a jar filled with tea and sugar solution
3	26°C	The SCOBY daughter formed is still very cryptic and very transparent
7	26°C	SCOBY's daughter is perfectly formed
10	26°C	SCOBY's daughter is 1 cm thick
12	26°C	pH 2
14	26°C	*daughter SCOBY 1.2 cm*

Evaluation of Fermentation Results

Evaluation of pH is one of the essential factors affecting kombucha fermentation. The acid content formed can affect kombucha activity. The resulting pH value is also related to microbial growth and changes in phytochemical compounds that affect antibacterial activity [4]. The results showed that the pH of the fermentation for 14 days resulted in the most acidic pH (2.87 ± 0.03). This is in accordance with the literature because the longer the fermentation time, the higher the organic acid produced, so the resulting pH is more acidic [20].

Fermentation Result Antibacterial Activity Test

The turmeric kombucha antibacterial activity test was carried out in vitro using the paper disc diffusion method (Kirby-Bauer Methode) against *P.acnes* bacteria by measuring the diameter of the inhibition zone in millimeters (mm). The results of testing the inhibition of kombucha turmeric 14 days, 10 days, and 7 days had inhibition against *P.acnes* bacteria. Turmeric kombucha fermented for 14 days with 15% turmeric concentration had the largest average clear zone of 8 mm, then 10% at 7.83 mm, and 20% at 7.33 mm. Kombucha turmeric 10 days 15% has the largest clear zone average of 8 mm, then 10% and 20% of 7.67 mm. Turmeric kombucha, fermented for 7 days with 20% turmeric concentration, had the largest average clear zone of 8.17 mm, then 10% was 8 mm, and 15% was 7.83 mm (Figure 2). The results obtained to follow the literature, namely, the longer the fermentation time, the more acidic the pH, thus affecting the antibacterial activity of kombucha [20]. The increasingly acidic pH is caused by the increased content of organic acids such as acetic acid; therefore, acetic acid acts as an excellent antibacterial agent in kombucha [19].

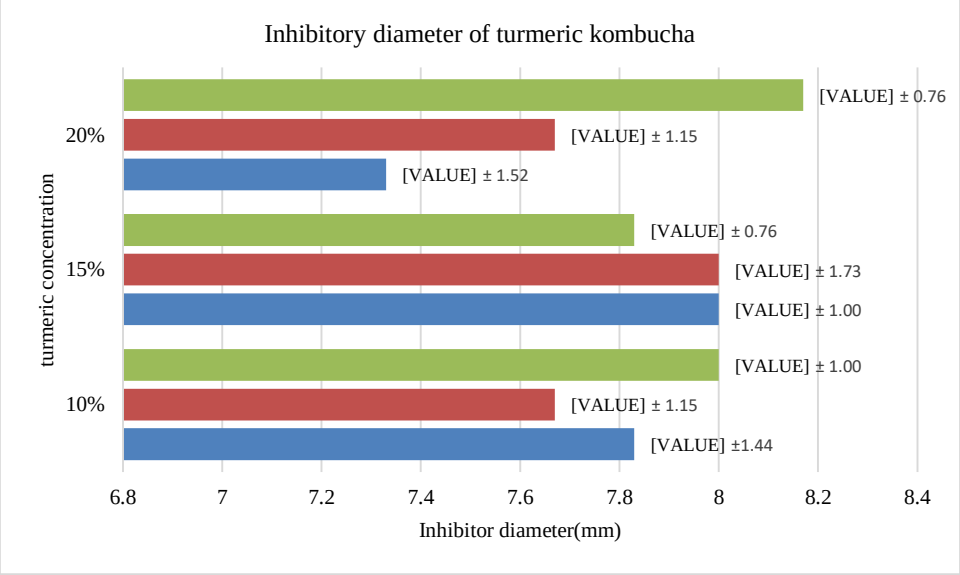

Figure 2: Diameter results of turmeric kombucha

(green: 7 days; red: 10 days; blue: 14 days)

The inhibition response of turmeric kombucha fermentation to *P.acnes* bacteria is relatively weak because it is 1 – 10 mm [21]. Based on the statistical results using the ANOVA test, the value of P <0.05 means that there is a significant difference between all treatments and positive controls. Post hoc statistical results showed P > 0.05, which means that the inhibition diameter of turmeric kombucha at 14 days, 10 days, and 7 days did not show any significant difference. Therefore, turmeric kombucha, used as an active ingredient in toner preparations, is taken from the smallest concentration, namely fermentation for 7 days with the addition of 10% turmeric.

Kombucha Turmeric Toner Formulation

The formulation of turmeric kombucha facial toner consists of turmeric kombucha, glycerin, propylene glycol, TEA, DMDM Hydantoin, and distilled water. Turmeric kombucha is an active substance that is expected to have antibacterial activity against the growth of *P.acnes* bacteria. Moisturizers prevent and treat dry skin by hydrating the stratum corneum, making the skin softer [22]. Glycerin and propylene glycol function as humectants, which work to absorb water from the atmosphere at >80% humidity and from the stratum corneum. [22]. Glycerin is one of the most widely used humectants because it can reduce the amount of water leaving the skin and moisturize the skin in high humidity conditions [23]. DMDM hydantoin is a preservative and one of the most widely used preservatives in cosmetics. This is because DMDM hydantoin is easily soluble in water, works as a broad-spectrum antimicrobial, and has a reasonably stable pH [24]. The safe concentration of DMDM hydantoin used in cosmetics is 0.1-1%. TEA (Triethanolamine) functions as an alkalizing agent that neutralizes turmeric kombucha's acidic pH.

Evaluation of Kombucha Turmeric Toner

Organoleptic Test

An organoleptic test was carried out by observing the preparation's aroma, color, and texture. The purpose of this test is to improve the quality of practice. Based on Table 3, the results of the organoleptic test can be seen that the turmeric kombucha face toner preparation is yellow; this is due to the effect of adding turmeric kombucha to the practice. The higher the concentration of turmeric kombucha added, the more cloudy the color will be. The aroma in all turmeric kombucha facial toner formulas produces a sour smell. The aroma comes from the fermented kombucha turmeric, and acetic acid is a compound that plays a role in the sour aroma and tastes like vinegar in kombucha [25]. All turmeric kombucha facial toner formulas have a liquid texture, are not sticky and are easy to absorb.

Table 2: Organoleptic test results

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

Formulas	Color	Aroma	Texture
F1	Transparent yellow	Turmeric and sour	Liquid, not sticky
F2	Slightly cloudy yellow	Turmeric and sour	Liquid, not sticky
F3	Slightly cloudy yellow	Turmeric and sour	Liquid, not sticky
F4	Colorless/clear	Not flavorful	Liquid, not sticky

Homogeneity Test

Homogeneity test was carried out to observe particles that were not mixed homogeneously in the formula. Mixing the active substance and additives in the toner preparation formula indicates the homogeneity of kombucha turmeric facial toner preparations. The results showed that all kombucha turmeric facial toner formulas did not produce insoluble particles. The addition of propylene glycol, which acts as a humectant and cosolvent, can increase the solubility of turmeric kombucha preparations.

pH test

The pH of turmeric kombucha facial toner was measured using a pH meter. Evaluation of the pH of kombucha turmeric facial toner preparations that meet the standards are F2 and F3 because they are in the range of 4.5 – 6.5 (figure 3) [17]. Based on the Kruskal-Wallis test results, the P <0.05 (0.015) value indicated a significant difference between the formulas for kombucha turmeric facial toner preparations. This happens because the concentration of turmeric kombucha as an active substance affects the pH of the preparation.

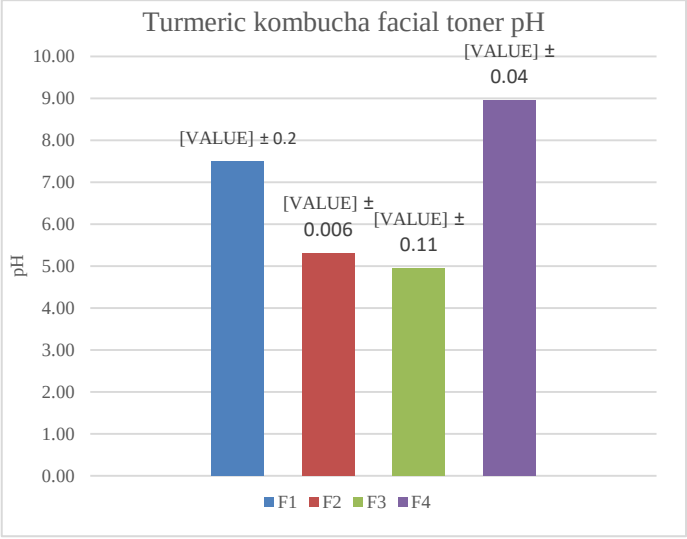

Figure 3: Evaluation of the pH of kombucha turmeric facial toner

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

Viscosity Test

Measurement of the viscosity of kombucha turmeric facial toner preparations using a Brookfield viscometer. The results of the viscosity of F1 were 13.46 ± 1.40 cPs, then F2 were 11.46 ± 0.83 cPs, F3 were 13.20 ± 0.40 cPs, and F4 were 11.20 ± 0.40 cPs (Figure 4).). Based on the results of the ANOVA test, the P value obtained for the viscosity test was P <0.05 (0.025), meaning that there was a significant difference between the formulas. All formulas have low viscosity values, indicating that the preparations contain well-dispersed particles and produce reasonable flow rates.

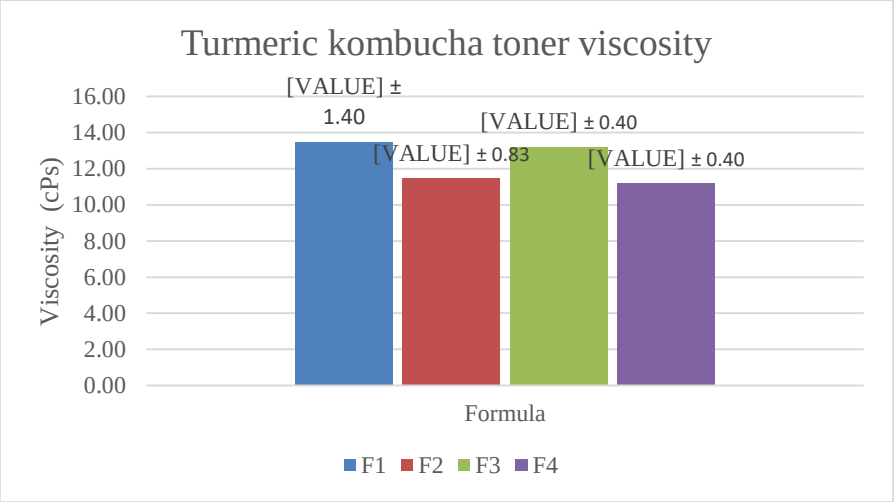

Figure 4: Viscosity results of kombucha turmeric facial toner

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

Room Temperature Stability Test

A stability test was carried out to determine the product's ability to maintain its properties and characteristics during storage at room temperature [26]. After the preparations were made, room temperature stability was observed for 28 days. Parameters monitored included organoleptic tests, pH tests, homogeneity tests, and viscosity tests. Organoleptic parameters were carried out by following aroma, color, and texture. All turmeric kombucha facial toner formulas do not change in aroma until the 28th day. The color produced by the turmeric kombucha facial toner changed to F1; on day 14, it changed color from transparent yellow to slightly cloudy yellow. Meanwhile, F2, F3, and F4 did not change color. The resulting F1, F2, F3, and F4 textures did not change for 28 days. The homogeneity parameter of turmeric kombucha facial toner preparations did not change; all formulas remained homogeneous until the 28th day. The pH parameters observed for 28 days changed each formula (Figure 5). F1 produced an increasingly acidic pH since day 14, whereas F2, F3, and F4 tended to be stable without experiencing a significant change in pH. This is presumably due to the low concentration of turmeric kombucha, which is 10%. During 14 days of storage, turmeric kombucha in the turmeric kombucha facial toner continues to metabolize so that the pH becomes acidic.

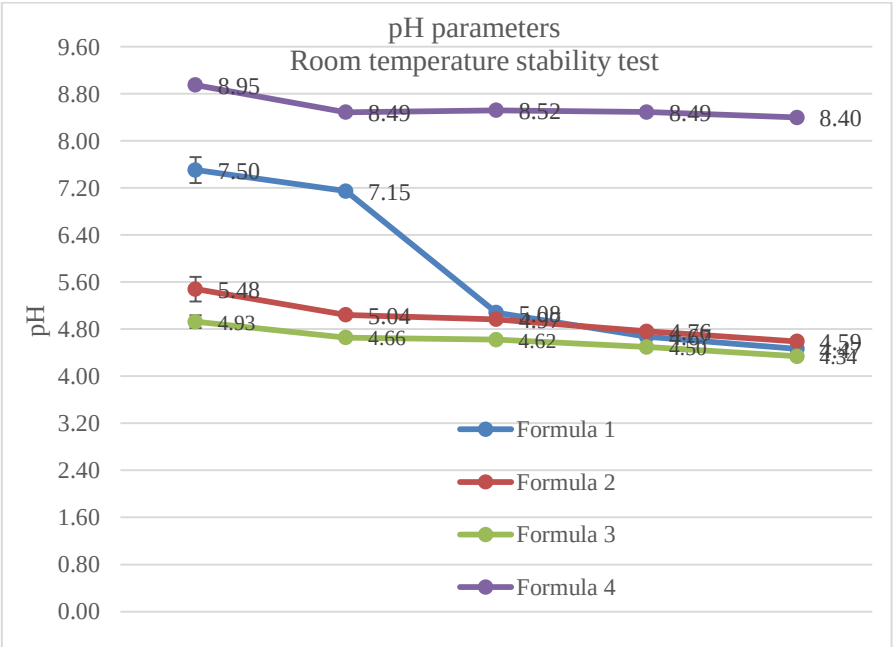

Figure 5: Results of pH stability test at room temperature for 28 days

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

The viscosity parameters of the kombucha turmeric facial toner for 28 days resulted in different viscosities (Figure 6). Based on the results of the ANOVA test, P value> 0.05 was obtained, and there was no difference in the viscosity values of F2 and F3 during storage at room temperature. So it can be said that F2 and F3 are stable.

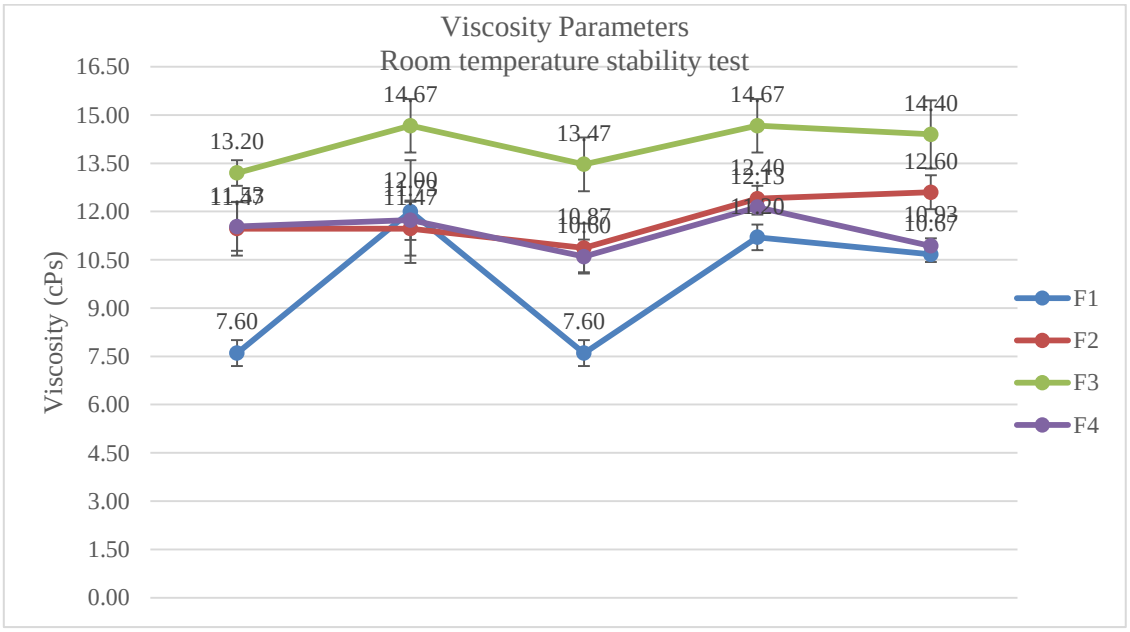

Figure 6: The results of the viscosity test for room temperature stability for 28 days

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

Stability Test Cycling Test

The stability test of the cycling test method was carried out by storing the preparations at ± 4°C and ± 40°C for 6 cycles. The parameters observed were organoleptic, homogeneity, and pH. Organoleptic parameters showed no change in aroma, color, and texture in all kombucha turmeric facial toner formulas from cycle 1 to cycle 6. Homogeneity parameters showed that all kombucha turmeric facial toner formulas were homogeneous from cycle 1 to cycle 6. The pH parameter in F1 experienced a decrease in pH starting in cycle 4. In F2, there was a decreased pH but not significantly, while in F3 and F4, the resulting pH tended to be stable (Figure 7).

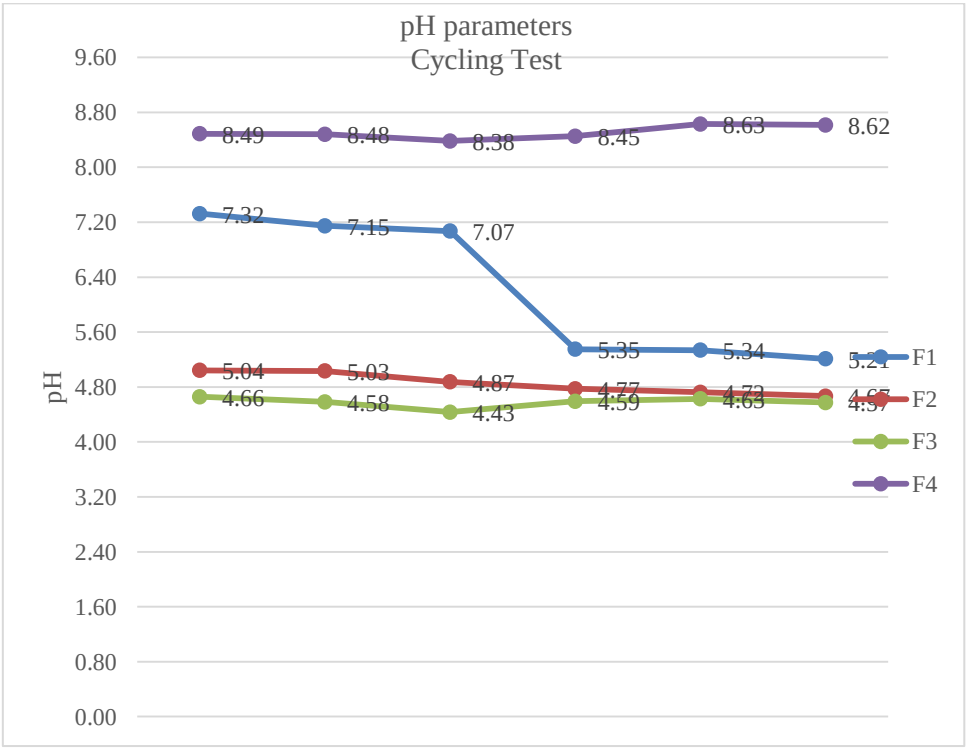

Figure 7: Results of pH measurements in the stability test of the cycling test method for 6 cycles

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base)

Irritation Test

The irritation test was carried out using the patch test method on 20 volunteers aged 18-25 years by placing the preparation on the forearm. After being observed for 48 hours, the test results showed that all of the kombucha turmeric facial toner formulas did not cause redness, itching, and swelling reactions in 20 volunteers. The results of the irritation test can be concluded that kombucha turmeric facial toner preparations are safe to use [27].

Kombucha Turmeric Toner Antibacterial Activity Test

After the preparation evaluation test, kombucha turmeric facial toner was tested for antibacterial activity against the *P.acnes* bacteria that causes acne. The turmeric kombucha facial toner was tested using the paper disc method, with the "X" brand kombucha toner already on the market as a positive control. Figure 8 shows that the brand "X" kombucha toner produces the largest diameter of 11 mm, followed by F3 with a diameter of 7.33 mm, then F4 and F1 have a diameter of 6.33 mm, and the minor diameter of F2 is 6 mm. F3 contains the highest turmeric kombucha, namely 20%; the high turmeric kombucha in facial toner preparations can produce a larger diameter compared to other formulas.

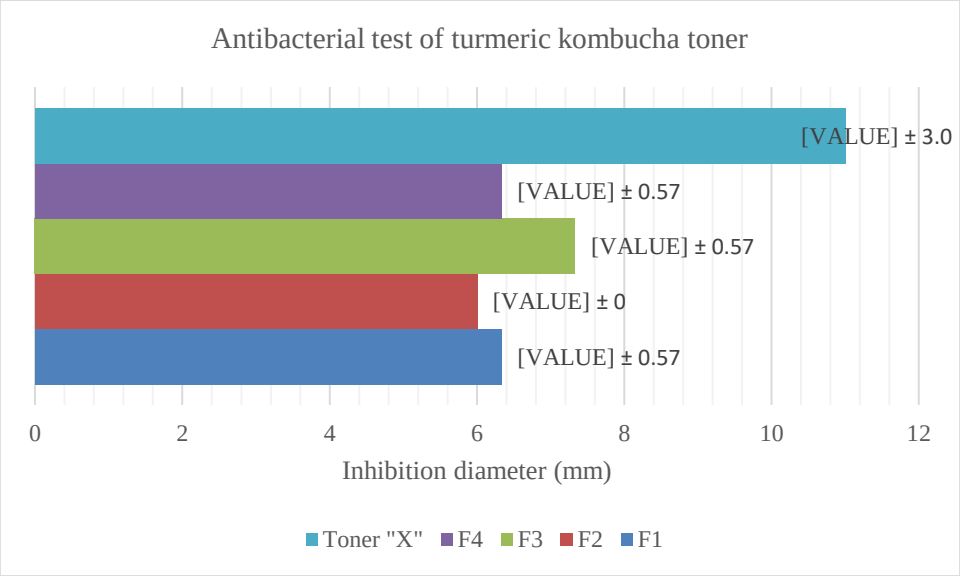

Figure 8: Results of the inhibition diameter of kombucha turmeric facial toner

F1 (10% turmeric kombucha), F2 (15% turmeric kombucha), F3 (20% turmeric kombucha), F4 (base), Toner "X" (kombucha toner on the market)

CONCLUSION

Based on the results of the study it can be concluded: The effects of fermented kombucha turmeric can be formulated into kombucha turmeric facial toner which meets the evaluation and stability test requirements; Turmeric kombucha facial toner has potential as anti-acne against *P.acnes* bacteria.

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