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Phytochemical Profile and Antibacterial Activity of Fermented Kombucha Black Tea (Camellia sinensis) Extract Against Acne-associated Bacteria

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Abstract: This study aimed to investigate the phytochemical profile and antibacterial efficacy of Kombucha black tea extract fermented for different durations (12, 18, and 20 days) against acne-causing bacteria. The research included qualitative phytochemical screening of alkaloid, flavonoid, saponin, steroid, and triterpenoid group identifications using specific assays. The study involved qualitative phytochemical screening and quantitative determination of total flavonoid content, expressed in milligrams of Quercetin Equivalents per gram of extract (mg QE/g). Antibacterial activity was evaluated using the disk diffusion method against *Cutibacterium acnes, Staphylococcus aureus*, and MRSA. Results showed that the extract is rich in flavonoids, phenols, saponins, and triterpenoids. The total flavonoid content varied significantly with fermentation time: 59.70 mg QE/g (Day 12), 80.28 mg QE/g (Day 18), and 71.95 mg QE/g (Day 20). The Day 18 extract demonstrated the strongest antibacterial inhibition against *C. acnes* ($p < 0.05$). In conclusion, optimized fermented Kombucha black tea extract exhibits significant antibacterial potential specifically targeting C. acnes, serving as a promising natural active ingredient for anti-acne therapy.

Keywords: Kombucha; Flavonoid; anti-acne; *C. acnes; S. aureus*.

INTRODUCTION

Acne vulgaris is a disorder of the pilosebaceous unit of the skin due to chronic inflammation with polymorphic clinical features in the form of seborrhea, comedones, papules, pustules, nodules, and cysts.[1] The predilection locations for acne vulgaris are the face, neck, back, and shoulders because, in these locations, the pilosebaceous glands are more numerous and larger.[2] The predilection for acne vulgaris is adolescence and can persist until the age of 30 years. Acne tends to be more common in men than women.[3] The peak period for acne vulgaris is 15-19 yea rs of age.[4] In other words, acne vulgaris is a common skin disorder and mainly affects teenagers. Reactive Oxygen Species (ROS) and oxidative stress play a role in the development of inflammatory acne lesions. To protect itself from ROS, the human body has an organized antioxidant system, which works synergistically. Antioxidants protect cells from oxidative damage and can prevent

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the production of oxidative products.[5] An imbalance between oxidants and antioxidants, where ROS production exceeds antioxidant capacity, has the potential to cause damage, called oxidative stress.

The pathogenesis of acne includes three factors, namely hyper seborrhoea, abnormal follicular keratinization, and proliferation of *C. acnes*.[6] The interaction of these factors changes the condition of the skin and triggers an inflammatory reaction.[7] In addition, the use of benzoyl peroxide, azelaic acid, and retinoids has side effects such as irritation. In contrast, long-term use can cause antibiotic resistance as well as organ damage and immune hypersensitivity.[8]. Irrational use of antimicrobials (antibiotics, antifungals) has caused many pathogenic microbes to adapt to their environment and become resistant to these drugs. The increasing problem of resistance causes the need for new antimicrobial drugs that can overcome the problem of resistance to increase. Therefore, the search for new antimicrobials, including those from plants, continues to be carried out. Acne treatment with antibiotics, benzoyl peroxide, azelaic acid, salicylic acid, and retinoids can be supplemented with herbal ingredients that have been empirically and clinically proven, such as black cumin oil and honey.[8]

Kombucha tea extract is a fermented tea made through a black tea fermentation process using a mixture of fungi and bacteria known as SCOBY (Symbiotic Culture of Bacteria and Yeast).[9,10] The fermentation process was carried out for 8 to 20 days. The antibacterial inhibitory effect against *C. acnes* was measured through the inhibition zone, namely the area around the paper disc where there was no growth of *C. acnes*. The content of flavonoid compounds in the n-hexane fraction can influence the nanoparticle nucleation process because flavonoid compounds are known as natural reducing agents and play a role in the nucleation process[11]. The benefits obtained from Kombucha include antibacterial and antioxidant [12]. Kombucha also has anti-inflammatory properties, which are very important for skin conditions and for preventing dermatological disorders[13]. Black tea contains various components such as phenolic acids, flavonoids (catechins), amino acids (theanine), methylxanthines (caffeine), lauric acid, β-carotene, lipids, carbohydrates, proteins, fluoride, volatile compounds, folate, vitamins A, C, and K.[14] The amino acid that is mostly contained in tea is theanine, which is 50%. The amino acids alanine and arginine cause the bitter taste of black tea[15].

Kombucha fermentation consists of two stages, namely aerobic fermentation and anaerobic fermentation.[16] Aerobic fermentation is the stage where sugar is converted into energy, CO_2, and H_2O. When oxygen runs out, anaerobic fermentation occurs17. The fungi or yeast in Kombucha consist of *Mycoderma, Zygosaccharomyces rouzii, Torulopsis, Candida famata, and Saccharomyces cerevisiae subsp. Cerevisiae, Pichia membranaefaciens, Brettanomyces bruxellensis, Schizosaccharomyces, Zygosaccharomyces bailii, Saccharomyces cerevisiae subsp. Aceti, Mycotorula, Brettanomyces intermedius,* and *Torulaspora delbrueckii*.[17] The kombucha bacterial colony consists of *Gluconobacter, Acetobacter xylinum, Acetobacter pasteurianus, and Acetobacter aceti*. Several factors influence fermentation, including pH, temperature, amount of oxygen, and fermentation time[18].

While Kombucha tea is widely recognized for its antioxidant and antimicrobial properties due to the presence of organic acids and polyphenols [19], limited studies have specifically investigated the effect of fermentation duration on flavonoid accumulation and antibacterial activity against *Cutibacterium acnes* the primary bacterium associated with acne vulgaris. Previous research has mainly focused on broad-spectrum antimicrobial activity, whereas the relationship between fermentation time, phytochemical dynamics, and anti-acne potential remains insufficiently explored. Therefore, this study evaluated black tea Kombucha fermented for 12, 18, and 20 days to determine the optimal

fermentation period for enhancing flavonoid content and antibacterial efficacy against acne-associated bacteria.

MATERIALS AND METHOD

Materials and instruments used in this study included standard laboratory equipment for fermentation, phytochemical screening, antibacterial assays, and spectrophotometric analysis. Black tea Kombucha was fermented under aerobic conditions at room temperature (25 ± 2°C). Samples were harvested on days 12, 18, and 20. These specific time points were selected based on preliminary screening which indicated that secondary metabolite production, particularly flavonoids and organic acids, fluctuates significantly during the mid-to-late fermentation phases[20].

Preparation of Kombucha Tea Extract was made by the following; black tea, distilled water, and granulated sugar were weighed and prepared. A sugar solution was made by heating water and sugar (ratio 1:150) with 50 grams of tea leaves added, heated, stirred, and cooled for 10 minutes. After filtration, 750 ml of filtered sweet tea extract was obtained and fermented in a glass jar with 10% kombucha starter for 8-21 days at room temperature.[21] Then the fermented kombucha filtrate was concentrated using a rotary vacuum evaporator until a crude extract was obtained. The mass of the resulting dry extract was precisely measured using an analytical balance. To achieve a standardized concentration of 100 mg/mL, the dry extract was then reconstituted by redissolving it in a specific volume of sterile solvent .The initial pH of the kombucha tea was 5.5, which decreased to a final pH of 4.0 by the end of the fermentation period.

Phytochemical tests included alkaloid, flavonoid, saponin, steroid, and triterpenoid group identifications using specific assays.[22] The total flavonoid content was determined using the aluminum chloride colorimetric method with Quercetin as the standard. The absorbance was measured at 371.5 nm using a UV-Vis spectrophotometer. The results were derived from a linear regression equation ($y = 0.0036x + 0.2452$, $R^2 = 0.999$) and expressed as mg Quercetin Equivalent per gram of extract (mg QE/g).

This study used the bacterial strains of *Cutibacterium acnes* ATCC 11828, *Staphylococcus aureus* ATCC 25923, and MRSA ATCC 33592. Antibacterial assay was prepared as follows: about 15 mL of Nutrient Agar (NA) was put into a sterile petri dish, then 100 µl of bacterial suspension (0.5 McFarland standard), and homogenized by shaking the petri dish. Allow the media to be hardened. Soak sterile discs according to the number of treatment groups, then place them on the solidified media.[23] The fermented extract was standardized to a concentration of 100 mg/mL. Each sterile paper disk was impregnated with 20 µL of the extract, resulting in a total extract load of 2 mg per disk. Sterile disks are soaked according to the number of treatment groups, namely: For the positive work control, Clindamycin 10 μg per-disk was used, while the others were used for three groups of black tea kombucha extract with the highest flavonoid content. Then, attach the soaked discs to the agar surface. The procedure was repeated three times. Incubate the Petri dishes in an incubator at 37°C for 24 hours, under anaerobic conditions. Then, the antibacterial activity was determined by measuring the diameter of the inhibition area with a caliper. All experiments were conducted in triplicate (n=3).

Data were analyzed using SPSS software. Normality of the data was verified using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test. Differences between groups were analyzed using One-Way ANOVA followed by a Post-Hoc Tukey HSD test. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of Kombucha Black Tea Extract

Organoleptic examination showed that the kombucha tea extract was a brownish liquid-like tea with a sweet and sour taste and a characteristic aroma of fermented tea. The representative of kombucha tea extract is shown in Figure 1.

Figure 1. Kombucha Black Tea Extract

Kombucha black tea extract generally has a darker color compared to kombucha tea from other types of tea. The color of black tea kombucha can vary depending on the processing process and concentration of sugar used. The aroma of black tea kombucha can vary but generally has a stronger and more complex aroma compared to Kombucha from other types of tea. This aroma was caused by microbial activity during the fermentation process. The taste of black tea kombucha usually has a more sour taste compared to Kombucha from other types of tea. The acidity level can vary depending on the length of fermentation and the type of tea used[24].

Phytochemical Screening of Kombucha Black Tea Extract

Qualitative screening confirmed the presence of flavonoids, phenols, saponins, triterpenoids, and steroids in the Kombucha black tea extract. Kombucha tea extract does not contain alkaloids as shown in Table 1.

Table 1. Phytochemical screening of fermented Kombucha black tea extract

Compound group	Reagent/test	Observation	Result
Flavonoids	Mg + concentrated HCl [20]	Reddish-orange	(+)
Phenols	$FeCl_3$ [20]	Blackish-green	(+)
Saponins	Distilled water + shaking (Forth test) [25]	Persistent foam for 30 sec	(+)
Triterpenoids & Steroids	Anhydrous acetic acid + concentrated H_2SO_4 [26,29]	Blackish-red	(+)
Alkaloids	Mayer and Dragendorff [28]	No color change / no precipitate	(-)

Quantitative analysis revealed that fermentation duration significantly influenced flavonoid levels as shown in the Figure 2. The highest total flavonoid content was observed on Day 18 (80.28 mg QE/g), followed by Day 20 (71.95 mg QE/g) and Day 12 (59.70 mg QE/g). The peak of total flavonoid content recorded on Day 18 represents the optimal fermentation window for the accumulation of bioactive metabolites. This peak is attributed to the maximal enzymatic activity of the SCOBY consortium, which optimally catalyzes the hydrolysis of complex tea polyphenols into simpler, more bioactive flavonoids (aglycones), thereby enhancing the chemical potency of the extract.[30] The subsequent reduction in flavonoid levels observed by Day 20 (71.95 mg QE/g) was likely indicative of over-acidification within the fermentation vessel. As the fermentation progresses beyond the optimal period, the excessive accumulation of organic acids creates a harsh environment that can trigger the degradation of sensitive bioactive compounds and polyphenolic structures.[31]

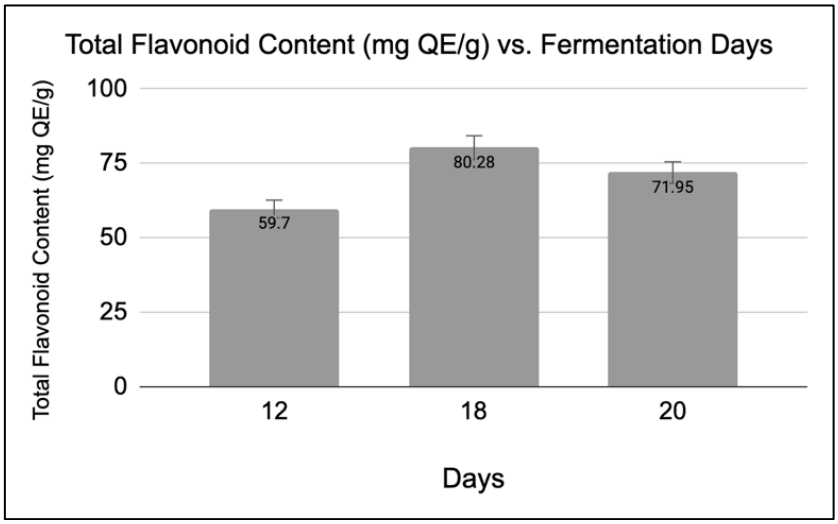

Figure 2. Total Flavonoid Content (mg QE/g)

Antibacterial Test of Kombucha Extract Formula

The formation of a clear zone around the paper disc demonstrated antibacterial activity, which can be seen from the results of the antibacterial test on the fermented black tea kombucha formula on days 12, 18, and 20. The bacteria used were *S. aureus*, MRSA, and *C. acnes*. The positive control sample was Clindamycin 10 *µ*g. Resistance measurements were carried out three times (triplicate).

Table 2. Inhibitory Effect of Kombucha Black Tea Extract against *S. aureus*, MRSA, and *C. acnes*

Bacterial Strain	Fermentation Day	Inhibition (mm) at Repetition			Mean Inhibition Zone (mm) ± SD	Inhibition Category
		1	2	3		
S. aureus	12	8.85	7.85	7.70	8.13 ± 0.62	Moderate
	18	7.57	8.22	10.85	8.88 ± 1.73	Moderate
	20	8.25	8.10	10.05	8.80 ± 1.08	Moderate
	Positive Control	24.05	24.05	24.05	-	Very Strong
MRSA	12	7.85	10.02	8.87	8.91 ± 1.09	Moderate
	18	9.70	7.40	10.37	9.15 ± 1.52	Moderate
	20	10.25	8.32	10.92	9.83 ± 1.33	Moderate
	Positive Control	20.87	20.87	20.87	-	Strong
C. acnes	12	10.00	9.40	10.00	9.80 ± 0.35	Moderate
	18	11.20	12.60	14.30	12.70 ± 1.59	Strong
	20	9.80	9.60	9.50	9.60 ± 0.15	Moderate
	Positive Control	23.57	23.57	23.57	-	Very Strong
Overall by fermentation day	12	-	-	-	8.95 ± 0.97	-
	18	-	-	-	10.25 ± 2.32	-
	20	-	-	-	9.42 ± 0.99	-

Note: Overall comparison by fermentation duration showed a significant difference in antibacterial activity among fermentation days (p = 0.022).

Table 2 demonstrates that the antibacterial activity of fermented Kombucha black tea extract varied depending on both the bacterial strain and fermentation duration. Overall, *Cutibacterium acnes* exhibited the highest susceptibility to the extract, particularly on Day 18 fermentation, which produced the largest inhibition zone (12.70 ± 1.59 mm) and was categorized as having strong antibacterial activity. In contrast, the inhibitory effects against *Staphylococcus aureus* and MRSA remained within the moderate category across all fermentation periods.

The overall mean inhibition analysis based on fermentation duration further showed that the Day 18 extract exhibited the highest antibacterial activity (10.25 ± 2.32 mm), followed by Day 20 (9.42 ± 0.99 mm) and Day 12 (8.95 ± 0.97 mm). Statistical analysis indicated a significant difference in antibacterial activity among fermentation periods (p= 0.022), suggesting that fermentation duration significantly influences the accumulation of bioactive compounds responsible for antibacterial efficacy.

The enhanced antibacterial activity observed on Day 18 may be associated with the optimal production of flavonoids, polyphenols, and organic acids during the mid-fermentation phase. These compounds are known to disrupt bacterial cell membranes, alter intracellular pH, and inhibit bacterial proliferation. The greater susceptibility of *C. acnes* compared to MRSA and *S. aureus* suggests that the fermented Kombucha extract may possess more selective antibacterial potential against acne-associated bacteria, supporting its possible application as a natural anti-acne agent. This higher sensitivity was rooted in the specific affinity of the kombucha's phenolic compounds for the cell membrane components of *C. acnes*, allowing for more effective penetration. In contrast, the relative resistance of MRSA was attributed to its notably thicker and more complex peptidoglycan layer, which serves as a more robust physical and chemical barrier against the penetration of bioactive flavonoids and organic acids.[32]

Table 3. Comparative Antibacterial Activity of Kombucha Black Tea Extract Against Different Bacterial Strains

Bacterial Strain	Mean Inhibition Zone (mm) ± SD	Overall ANOVA p-value	Pairwise Comparison	Post-hoc p-value
Staphylococcus aureus	8.60 ± 1.12		vs MRSA	0.881
MRSA	9.30 ± 1.23	0.011*	vs *C. acnes*	0.118
Cutibacterium acnes	10.77 ± 1.69		vs *S. aureus*	0.010*

Notes: Values are presented as mean ± standard deviation (SD). Statistical analysis was performed using One-Way ANOVA followed by Tukey HSD post-hoc analysis. *p < 0.05 indicates statistical significance.

The comparative analysis (table 3) demonstrated a statistically significant difference in the antibacterial activity of fermented Kombucha black tea extract among the tested bacterial strains ($p < 0.05$). *Cutibacterium acnes* exhibited the highest susceptibility, with a mean inhibition zone of 10.77 ± 1.69 mm, followed by MRSA (9.30 ± 1.23 mm) and *Staphylococcus aureus* (8.60 ± 1.12 mm). Post-hoc analysis revealed a significant difference between *C. acnes* and *S. aureus* (p = 0.010), whereas no significant differences were observed between MRSA and the other bacterial strains. These findings suggest that the fermented Kombucha extract possesses greater antibacterial efficacy against acne-associated bacteria, particularly *C. acnes*.

Black tea kombucha produces acetic acid during the fermentation process. This acetic acid has strong antibacterial properties, which can inhibit the growth of gram-positive and negative bacteria. Black tea contains polyphenols which have antimicrobial activity. During the fermentation process, these polyphenols accumulate in Kombucha, increasing its antibacterial potential.[16] Fermentation time also affects antibacterial activity.[21]

The antibacterial activity may be associated with the presence of organic acids, polyphenols, flavonoids, and other bioactive compounds that can disrupt bacterial membrane integrity or interfere with bacterial growth. These compounds include aflavins, arubigin, polyphenols, and organic acids such as acetic acid and glucuronic acid.[33]

The inhibitory effect against MRSA is in the moderate range. This is because MRSA is a form of *S. aureus* that is resistant to methicillin, which has thicker and more complex cell walls than ordinary *S. aureus*. The peptidoglycan in

MRSA cell walls is more difficult for antibiotics to destroy, which makes them more resistant to antibiotics.[25]

To compare with previous studies, our findings corroborate the work that reported that the antioxidant potential of Kombucha fluctuates with fermentation time.[19] Similarly, other study observed that specific SCOBY interactions enhance phenolic content.[20] Unlike broad-spectrum studies, our results highlight a specific susceptibility of *C. acnes* to the extract compared to *S. aureus*, suggesting a targeted potential for acne therapy.

While the disk diffusion results indicate significant inhibitory zones, further studies are required to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values to fully characterize the antibacterial potency of the extract. Clinical implications suggest that Kombucha black tea extract could serve as a viable alternative to conventional antibiotics like clindamycin, reducing the risk of antibiotic resistance while providing antioxidant benefits to acne-prone skin.

CONCLUSION

Fermented kombucha tea extracts from days 12, 18, and 20 demonstrated a significant presence of flavonoids, phenols, saponins, and steroids/triterpenoids, while alkaloids were absent. The flavonoid content in these extracts ranged from 59.70 mg QE/g to 80.278 mg QE/g, indicating substantial flavonoid enrichment. The black tea kombucha extract exhibited significant antibacterial activity against *C. acnes*, with moderate effects observed against *S. aureus* and MRSA. Notably, the antibacterial activity against *C. acnes* was notably stronger compared to *S. aureus*. Statistical analysis confirmed that the duration of fermentation significantly influenced the antibacterial efficacy, with the Day 18 extract demonstrating the most potent inhibitory effect against *C. acnes* ($p < 0.05$). These findings underscore the potential of black tea kombucha extract as a promising source of bioactive compounds with notable antimicrobial properties, particularly effective against *C. acnes*.

AUTHORS' CONTRIBUTIONS

Larastika Yovianda: Data curation, Investigation, Writing- Original draft preparation.: Merisca Gianthra Ryosa: Data curation, Investigation.: Ully Chairunisa: Data curation, Investigation.: Erny Tandanu: Supervision, Methodology, Validation.: Refi Ikhtiari: Supervision, Conceptualization, Review and Editing.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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