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Development of Serum Stick from *Medusomyces gisevii* (kombucha) to Restore Facial Skin Assets

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ABSTRACT

Kombucha, a fermented tea drink, is praised for its potential health benefits and antioxidant properties. Although studies suggest that kombucha has antioxidant properties, this trend is somehow inconsistent (some journals show that the antioxidant content is lower during fermentation while others show the opposite). This inconsistent research creates a gap in knowledge about the effectiveness of kombucha for topical applications such as wrinkle reduction and increased elasticity. Additionally, traditional liquid serums present challenges in travel and use. This research study investigated the potential of kombucha as an anti-aging agent in skin care, specifically in the form of a serum stick, and found that the antioxidant content in kombucha filtrate increased with fermentation time (comparison between day 7 and day 17), with the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) values observed on day 17, specifically 2411.7647 mg GAE/L and 600 mg QE/L, respectively. The DPPH test confirmed this finding, showing a stronger antioxidant effect on day 17 (lower IC_{50} value) of 28.0178 mg/L. The antioxidant properties of kombucha are attributed to the presence of bioactive compounds such as polyphenols, and flavonoids, which have been shown to neutralize free radicals, reduce oxidative stress, and promote collagen synthesis, ultimately leading to better skin elasticity and reduce lines and wrinkles. The serum stick formulation incorporating kombucha filtration has been developed. Patch testing showed good tolerance across different testers. Users experience testing (organoleptic testing) identified areas for improvement regarding product smoothness and ease of use, with

formulation 2 found to be superior to formulation 3.

Keywords: Kombucha; DPPH; TPC; TFC; Antioxidant

INTRODUCTION

Although fermented foods have been a staple of cultures internationally for thousands of years, kombucha has only recently become popular in some countries including the United States and Indonesia. Not only that, the kombucha market in Asia-Pacific (APAC) is projected to see a 20% jump between 2019 and 2025, driven by enthusiastic consumers in places like Australia, Hong Kong, the Philippines, and Malaysia [1]. In fact, Malaysian brand Wonderbrew saw sales almost double within one year (in 2020). This shows the great acceptance and response by Malaysians towards kombucha. Kombucha is now popular among consumers worldwide because it contains many benefits that fit the "healthy trend" pattern derived from the activity of a consortium of acetic acid bacteria and osmophilic yeast, called "tea fungus". It has gained popularity in the West because of its therapeutic effects, such as antimicrobial, antioxidant, anticarcinogenic, and antidiabetic, treatment for gastric ulcers and high cholesterol, and it has also shown effects on immune response and liver detoxification. The beneficial effects of kombucha are attributed to the presence of bioactive compounds that act synergistically [2].

Other than the benefits offered by kombucha, the flavour which is slightly acidic and slightly carbonated also plays an important role which provides greater acceptance among the consumers. To make kombucha, a drink made from the fermentation of tea, only a few ingredients are needed with some simple steps or procedures that must be followed. Generally, tea (black or green tea), sugar, and a Symbiotic Culture of Bacteria and Yeast (SCOBY) are needed, and they must be fermented for 7 to 10 days. If the fermentation time is longer, the amount of bioactive compound will increase. Bioactive compounds that are present in kombucha are the genus *Acetobacter, Gluconobacter*, and the yeasts of the genus *Saccharomyces* along with glucuronic acid that contribute to health protection [2]. Because of the advantages of kombucha, it has been studied for use in skin care. When it comes to skincare routines, consistency is the key. So that, the

caliber of the product can be seen as expected by the user. In terms of serum, it is one of the skincare products that should be used on the skin because it improves the condition of the skin with a significant reduction in the surface area of the skin occupied by micro folds and in the roughness of the skin. Dermatological evaluation of the skin concluded that there was a statistically and clinically significant improvement in skin smoothness, wrinkle severity, visibility of fine and raised lines, tightening effect in the crow's feet, forehead, and upper lip areas with the application of the serum to the skin. Therefore, this study will focus on the antioxidant content in kombucha that can help reverse the effects of aging and improve the condition of the skin in the form of a serum.

Fermentation is a bioprocess widely used to produce biologically active compounds, primarily in the food industry, but recently, it has been increasingly used in cosmetics to improve the quality of active phytochemicals and to facilitate the absorption of these substances by the human system. However, scientific studies on *Medusomyces gisevii* (kombucha) on the skin (skincare) are too few, especially for serum products. Although there is a lot of scientific research on antioxidants in kombucha, there are inconsistencies in the trend of antioxidant content during fermentation found in previous studies after comparing several journals [3-8]. Cytotoxicity was determined on keratinocyte and fibroblast cell lines, resulting in a significant increase in cell viability for the ferments. This antioxidant potential of the obtained fermentation was analyzed by evaluating the scavenging of external and intracellular free radicals. Fermentation shows a strong ability to inhibit the activity of lipoxygenase, collagenase, and elastase enzymes and long-lasting hydration after its use on the skin. This suggests that kombucha holds significant promise for promoting skin health. Its strong antioxidant activity, ability to support cell growth, enzyme-inhibiting properties, and long-lasting hydrating capabilities position it as a potentially valuable addition to skincare routines. While further research is necessary to understand and optimize their benefits fully, these findings pave the way for the development of novel and effective skincare solutions based on fermented ingredients.

To ensure that most people can apply and reapply serum on time or when needed on the face, the serum stick is the solution. Traditional face serums are usually not travel-friendly. Most are prone to leaks and the glass bottles used to fill the serum are a bit heavy and too delicate to fit in a tote

bag or luggage. Not only that when it comes to Standard and Transportation Security Administration (TSA) Pre-Check Screening at the airport, people can carry only 100 mL of liquid in their bag. This will give additional work because they must repack their skincare items if they exceed the limit. So, when this serum stick alternative comes, this problem can be solved easily.

Although kombucha shows promise as an anti-aging agent with the presence of polyphenols, vitamin C, and probiotics, scientific evidence regarding specific effects on the skin (skincare) is currently limited. Research on kombucha as an anti-aging serum has the potential to contribute to the cosmetic field. This could lead to the development of new anti-aging formulations that cater to consumer demands for natural and holistic skincare options. Additionally, understanding antioxidant trends during fermentation and the specific amount of antioxidant contents found in kombucha that contribute to anti-aging effects can inspire further research and innovation in the cosmetics industry. This knowledge could lead to the development of novel ingredients or technologies that mimic or enhance the effects of kombucha, resulting in more advanced and effective anti-aging products. The skin serves as a protective barrier between the body and its surroundings, maintaining hydration and preventing the entry of harmful microorganisms. Additionally, the skin plays a significant role in aesthetic appearance. To combat the visible signs of aging, skincare routines can be one of the strategies to protect the skin against environmental stressors, strengthen the skin barrier, and maintain a balanced skin microbiome. In a skincare routine, a few steps need to be followed. To make it short and vivid, the basic daily skincare routine is cleansing, moisturizing, and applying sun protection. However, for a more comprehensive and impactful approach, exfoliation, toning, serum treatments, and eye creams also need to be considered in the skincare regimen.

Skin aging is a captivating biological concert, where the once vibrant canvas of youth turns into a tapestry of time nuances. Cutaneous structure and physiological changes are hallmarks of skin aging [9]. This metamorphosis is regulated by a complex interaction of intrinsic and extrinsic factors, subtly altering both the structural integrity and physiological function of the precious integument. Phenotypic changes in cutaneous cells and structural and functional changes in extracellular matrix components like collagen, elastin, and proteoglycans, are necessary to give the skin its tensile strength,

elasticity, and moisture, respectively. Based on Gu *et al.* [10], intrinsic aging regulated by genetic factors affects all areas of the skin. This can lead to the ineffectiveness of serum use as it is caused by natural processes in the body, such as telomere shortening and cellular aging, and cannot be reversed by topical treatments such as serum. In this study, the focus will be on treating and preventing the effects of extrinsic aging. The onset of dyschromia, roughness, and fine wrinkles, which are followed by deep and lasting folds, are signs of external aging.

Based on Barakat [11], kombucha fermentation is a complex microbial interaction. Initiated by *Acetobacter xylinum*, a cellulose matrix is formed, providing the structural basis for subsequent microbial activity. The yeast organism then catalyzes the conversion of sucrose into glucose and fructose, simultaneously producing ethanol. In the next stage, acetic acid bacteria convert these simple sugars into organic acids, namely glucose into gluconic acid and fructose into acetic acid. Based on a study conducted by Wang *et al.* [12], the main bioactive components in tea are flavonoids and polyphenols. The amount of flavonoids and polyphenols increases as the fermentation time increases. This statement has been strengthened by Morales [13] who has stated that polyphenols are the primary source of antioxidant activity in kombucha. However, other components such as ascorbic acid also play significant roles, creating a multifaceted and effective antioxidant system. Meanwhile for the phenolic compounds present in kombucha, flavonoids, especially catechins, are the main ones. The superior antioxidant activity of kombucha compared to unfermented tea is due to the combined effect of two factors which are enhanced polyphenol activity and the presence of additional antioxidant compounds from black tea. Because of their ability to scavenge free radicals and ROS, polyphenols that make up about 30% of the total dry weight of fresh tea leaves are considered crucial compounds. Then, it can be concluded that polyphenols, flavonoids, catechins, and ascorbic acid are among the confirmed bioactive compounds in fermented kombucha, responsible for its powerful antioxidant properties.

EXPERIMENTAL METHODOLOGY

Preparation of Kombucha Fermented Tea Solution

The kombucha fermented tea has been prepared. The ratio of water, sugar, black tea, and kombucha solution was measured based on the percentage ratio prepared by Kaewkod *et al.* [14] which is 100:10:1:10 respectively with some modifications on the amount of tea and sugar used (as shown in Table 1). 1000 mL of water has been boiled, and 2 g of black tea was added (1 tea bag). This was done in 15 minutes. Then, 150 g of sugar was added to the hot tea. The mixture was allowed to cool at room temperature. A recent study suggested that the kombucha fermentation temperature was 28 °C and the optimum temperature for fermentation of black tea kombucha was 28 ± 2 °C. The cooled mixture then has been transferred into a sterilized container that has a wide bottleneck to allow easy access and enough surface area for air exchange with the environment. To ensure the viability of the beneficial bacteria in the kombucha, the sweet tea mixture was cooled to room temperature before carefully introducing 100 mL of kombucha starter solution to initiate the fermentation process. The container was covered with porous fabric for ventilation and contamination prevention. Only one jar or container was used at first (original solution), and to stop the fermentation, a small amount of kombucha fermented tea solution was transferred from the main jar into the smaller sterilized container that was sealed tightly and put into the refrigerator (at 5 °C) to prevent further fermentation. Fermentation was stopped on days 7 and 17. The sample was filtered through a 0.45 μm membrane and prepared for further analysis.

Table 1: Preparation of kombucha fermented tea solution.

Specifications	Details
Water	1000 mL
Sugar	150 g
Black tea	2 g
Kombucha starter solution	100 mL
Cool temperature	25 ± 5 ˚C
Fermentation time	7-17 days

Preparation of Kombucha Stock Solution

To make a 100 ppm kombucha stock, 0.01 mL of kombucha was taken from the container using a micropipette (10 µL) into a 100 mL volumetric flask. Then, solvents (methanol for DPPH and distilled water for TPC and TFC) were added to the calibration mark. The concentration was calculated first using the v/v % formula in Equation (1).

$$\text{Percent by volume } \left(\frac{v}{v}\right) = \frac{0.01 \text{ mL}}{100 \text{ mL}} \text{ x } 100 \quad (1)$$

From this formula, 0.01% v/v was obtained. It was then converted to ppm concentration units to standardize the units used in this study. To convert v/v% to ppm, 0.01% has been multiplied by 10000. Therefore, 0.01% (v/v) is equal to 100 ppm.

Determination of Total Phenolic Content (TPC)

Kombucha fermented tea's TPC was quantified using the steps applied by Molole *et al.* [15] with some modifications. 1000 mg/L of kombucha liquid was prepared with distilled water. 200 µL of Folin-Ciocalteu reagent (2 N) was added to the sample (0.4 mL). Then, the mixture has been incubated for 8 minutes at room temperature. After that, 600 µL of sodium carbonate (Na_2CO_3) solution (75 g/L in water) was added. The mixture was incubated for another 30 minutes, allowing the reaction to proceed further. For the blank, distilled water was used to replace the sample. After incubation, the absorbance of the solution was measured at 760 nm using a UV-Vis spectrophotometer. A standard calibration curve has been prepared using the same procedure with different concentrations of gallic acid (a reference standard for phenolic content) which are 10, 50, 70, and 100 µg/mL. Blank containing all reagents except gallic acid was used. By comparing the absorbance of the kombucha samples to the calibration curve, the concentration of phenolic compounds in the samples was determined, expressed as milligrams of gallic acid equivalents (GAE) per liter of kombucha.

Determination of Total Flavonoid Content (TFC)

Kombucha fermented tea's TFC was quantified using the method applied by Alam and Sharma [16] with some modifications. Quercetin was used as a standard for this test with concentrations of 15, 50, 100, and 200 ppm. Blanks containing all reagents except quercetin have been used. 0.1 mL of quercetin was taken and mixed with 0.5 mL of distilled water, and 0.1 mL of 5% sodium nitrate ($NaNO_2$). The mixture was then left for 6 minutes. Then, 0.15 mL of 10% aluminum chloride ($AlCl_3$) was added and left for another 5 minutes. After that, 0.2 mL of 1 M sodium hydroxide (NaOH) was added as a complement to this method. The absorbance was measured at 510 nm. All procedures were repeated for samples. For the blank, distilled water was used to replace the sample. By comparing the absorbance of the kombucha sample to the calibration curve, the concentration of flavonoid content in the sample was determined, expressed as milligrams of quercetin equivalents (QE) per liter of kombucha.

Determination of DPPH Scavenging Capacity

To determine the number of antioxidants in kombucha fermented tea solution (sample), a DPPH test was done. The method that has been used is basically from Nerdy and Manurung [17] with some changes. A 40 ppm DPPH solution was prepared to be added to the diluted sample solution (from a 100 ppm stock) resulting in different concentrations of 10, 20, 30, 40, and 50 μg/mL diluted with methanol. The concentrations used for the ascorbic acid standards were 10, 20, 30, and 40 μg/mL. The ratio of the DPPH solution added into the diluted sample is 4:1 (same as the ratio for DPPH and ascorbic acid standard) which has been mixed in the test tubes. By analyzing kombucha at different concentrations, the inhibitory concentration (IC) at which kombucha scavenges 50% of DPPH radicals was determined. The mixture has been incubated for 30 minutes at 25 °C in the dark. A DPPH solution without a sample added has been used as a control. While the blank used is methanol. The absorbance was measured at 513 nm by using a UV-Vis spectrophotometer. An ascorbic acid solution was prepared to act as a standard solution (a known antioxidant) as a reference for comparison. DPPH inhibition percentage was calculated using Equation (2).

$$\% \text{ inhibition} = ((A_0 - As) / A_0) \times 100 \quad (2)$$

where:

A_0 —absorbance of DPPH solution at 513 nm without tested sample
As —absorbance of DPPH solution at 513 nm with tested sample
After that, the IC_{50} was determined based on the graph obtained from the DPPH test. IC_{50} represents the concentration required to achieve 50% inhibition of the target activity being studied.

Development of Serum Stick

Medusomyces gisevii (kombucha) serum stick, was made by using the formula for the balm stick prepared by Dwita *et al.* [18] that has been utilized to make the *Nigella sativa* balm stick (Figure 1) with some modifications. The new formula that has been applied is shown in Table 2.

Notes: Taken from Dwita *et al.* [18].

Figure 1: *Nigella sativa* Balm Stick.

Table 2: Formulations of Medusomyces gisevii (kombucha) serum stick.

Materials	Function	Amount (mL)		
		Control (F1)	F2	F3
Medusomyces gisevii (kombucha)	Active substance	0	2	3
Cera Alba (Beeswax)	Hardener	4	3	3
Olivem 1000	Emulsifier	0.1	0.1	0.1
Virgin coconut oil (VCO)	Emollient	3	2	1
Glycerin	Humectant	1	1	1
Lavender essential oil	Perfume	1 drop	1 drop	1 drop
Total volume (mL)		8.1		

Serum sticks with varying *Medusomyces gisevii* (kombucha) liquid content have been produced in a multi-step process. First, beeswax was melted in a water bath at 65 °C, then combined with Olivem 1000 until a homogeneous mixture formed. This liquid wax mixture was then blended with the VCO solution before finally adding the desired percentage of *Medusomyces gisevii* (kombucha) which has been mixed with glycerin first. By referring to F2 (formulation 2), 1 mL of glycerin, 2 mL of *Medusomyces gisevii* (kombucha), 3 mL of cera alba, 0.1 mL of Olivem 1000, and enough amount of VCO which is 2 mL was added to reach a total weight of 8.1 mL. Then, a drop of perfume was added. The complete mixture was then poured into a mold and allowed to solidify, forming the finished serum stick. For optimal storage, the serum sticks were stored at room temperature. A control serum without any added liquid *Medusomyces gisevii* (kombucha) provided a neutral baseline for comparison. F2 and F3 had different concentrations of *Medusomyces gisevii* (kombucha) liquid to compare the effects of different doses of *Medusomyces gisevii* (kombucha) and the best formulation was found.

Physical Test on Serum Stick

The pH test was carried out by dipping the pH meter into a mixture of serum that has been diluted with distilled water.

Patch tests were conducted to assess potential skin inflammation and allergies. The serum stick was applied to the skin and monitored for adverse

reactions. Skin irritation tests were performed to evaluate the formulation's irritating potential. The product, containing varying concentrations of *Medusomyces gisevii* (kombucha), was applied to the skin for 30 minutes. A total of 10 participants were recruited through convenience sampling. Participants were healthy adults aged 20-40 with normal to oily skin and no history of skin allergies. Exclusion criteria including pregnancy, breastfeeding, current skin conditions, and use of topical medications were considered before selecting participants.

Organoleptic testing is a scientific assessment of the sensory properties of a product using the five senses that are appropriate for consideration. For this study, it focused mainly on observations of changes in smell, shape, colour, homogeneity, and texture. This has been done for all serum sticks during storage at room temperature on days 1, 3, 5, and 7.

RESULTS AND DISCUSSION

Kombucha Fermentation

Kombucha fermentation is a captivating process characterized by a significant transformation in the beverage's chemical composition. Several key factors demonstrably influence the final product, including the type of base tea, initial sugar and tea leaf quantities, fermentation duration, and incubation temperature. The type of base tea, with its unique flavour profile and nutrient content, significantly impacts the final product. The initial quantities of sugar and tea leaves are crucial for balancing sweetness and strength. Fermentation duration determines the development of flavours and acidity, while incubation temperature regulates the fermentation rate. These variables interact dynamically, making kombucha production a delicate process that requires careful control to achieve desired taste profiles and product consistency. As an example, employing a tea rich in antioxidants can potentially yield a more potent final product, while adjusting fermentation time directly impacts the kombucha's acidity (tanginess). Furthermore, Kitwetcharoen *et al.* [19] highlight the significant influence of the initial tea leaf quantity on the final composition. These leaves act as a source of unique chemical elements, impacting the concentration of antioxidants

(like catechins), caffeine, and other flavour precursors derived from the tea itself. A higher quantity can promote a more vigorous fermentation process.

However, excessive tea leaf concentration (above 6 g/L) can inhibit acetic acid bacteria growth, consequently reducing cellulose production. Cellulose is the primary component of the SCOBY. As the SCOBY metabolizes sugar, it produces cellulose, forming a protective matrix that houses the microbial community. This process is essential for SCOBY growth and development, and its inhibition can adversely affect kombucha fermentation. This cellulose contributes to the SCOBY's thickness and its protective role for the underlying bacteria and yeast. This can be seen throughout the fermentation process carried out in this study. The SCOBY continued to thicken day by day (observations were made up to day 24). Kombucha fermentation relies heavily on starter tea, a prior batch's acidic liquid. This starter tea rapidly reduces the pH of freshly brewed sweet tea, creating an environment that suppresses unwanted bacteria while favouring the growth of beneficial microbes (pH below 4.5 is needed). Low pH levels kill harmful bacteria while keeping the healthy bacteria and SCOBY alive. As these microbes consume sugars, the pH further decreases, signifying active fermentation. The resulting finished kombucha typically possesses a pH between 2.5 and 3.5, contributing to its signature tangy flavour. Therefore, pH serves as a crucial indicator throughout the fermentation process, from the starter tea's initial influence on the final beverage's characteristic acidity.

In this study, a more acidic starter was suspected to be used because of the pH measurements on day 7 and day 17 which were slightly lower than the range stated before (as shown in Table 3). The reliability of these findings is further bolstered by the consistency observed in related research. Chakravorty *et al.* [20] reported a final pH of 2.28 after 7 days of fermentation. While Jakubczyk *et al.* [3] documented a pH of 2.31 on day 17. It is not only that, in some studies, the pH decreased rapidly from 5.0 to 3.0 within 3 days of fermentation and continued to decrease slightly up to 18 days. This convergence in results across different studies strengthens the credibility of the observed pH decrease during kombucha fermentation.

Table 3: pH measurement for different fermentation days of kombucha.

Fermentation day	pH
7	2.498 (acidic)
17	2.135 (acidic)

This investigation into kombucha fermentation yielded several noteworthy observations. The formation of a new SCOBY layer by day 5 aligns with established timelines for kombucha brewing. A new layer of SCOBY, often referred to as a "daughter SCOBY," developed on the kombucha's surface due to the collaborative efforts of Gluconobacter, Acetobacter, yeast, and other microorganisms within the liquid as shown in Figure 2 and 3 (side and upper look).

Figure 2: Fermented kombucha (day 17).

Figure 3: New SCOBY.

The daughter SCOBY gradually thickened as these organisms multiplied and produced cellulose. Furthermore, the measured density of 1 g/mL coincides with the findings of Coton *et al.* [21], for black tea kombucha. An intriguing observation was the colour shift from dark to light as fermentation progressed. This observation is in line with the findings reported by Jakubczyk *et al.* [3], who documented a continuous increase in polyphenols, especially flavonoids, and the transformation of thearubigin (contributing dark colour) to theaflavin (contributing lighter colour) during kombucha fermentation. However, it is important to acknowledge that some studies report a slight darkening of kombucha during fermentation. This variation could be attributed to factors like catechin oxidation, as suggested by Nguyen *et al.* [22]. This oxidation process can contribute to a darker colour and distinct flavour in the final product. These observations highlight the dynamic changes occurring during kombucha fermentation and the potential influence of various factors on the final product's characteristics.

Lastly, the aroma of kombucha is a dynamic process shaped by microbial activity during fermentation. Initially, the tea base imparts floral and sweet notes. As fermentation progresses, various volatile organic compounds (VOCs) such as alcohols, acids, and esters, transform the flavour profile. *Gluconobacter* and *Acetobacter* contribute tartness and vinegar-like notes respectively. As observed, on the 3rd day sour kombucha can be smelled contributed by carboxylic acids, while compounds such as 2-phenylethanol introduce sweet and floral nuances.

DPPH Assay

The DPPH assay predicts antioxidant activity by measuring their free radical scavenging capacity. This assay utilizes the ability of antioxidants to inhibit lipid oxidation through DPPH radical neutralization. To clarify further, DPPH molecules, which are unstable free radicals, are neutralized into a stable form through the donation of a hydrogen atom from antioxidant molecules present in the sample or standard. The higher the number of DPPH molecules neutralized by a given amount of antioxidants, the stronger the antioxidant's free radical scavenging ability. For the DPPH test, ascorbic acid has been used as a standard. The ascorbic acid has been prepared for 10 ppm, 20 ppm, 30 ppm, and 40 ppm. This positive control is essential for the

comparison of the sample and the known antioxidant. Methanol was used as a blank while 40 ppm DPPH acted as a negative control which gives the absorbance of DPPH before scavenging by standard or sample. The highest standard concentration of 40 ppm ascorbic acid was screened to obtain the appropriate wavelength that could be used by referring to the highest absorbance that appeared. 513 nm is the best wavelength that has been chosen. It was in the absorbance wavelength range (510–520 nm) reported in the literature for DPPH solutions using UV-Vis Spectrophotometer [23]. The negative control's absorbance also has been determined at the same wavelength. For this test, the higher the concentration, the lower the absorbance value obtained, indicating stronger antioxidant activity. The colour of the mixture reduced from violet to yellow as shown in Figure 4. It is a visual indicator of the antioxidant activity in the standard and sample. The greater the colour change (more yellow), the more effectively the sample can neutralize free radicals. The standard curve of ascorbic acid is shown in Figure 5.

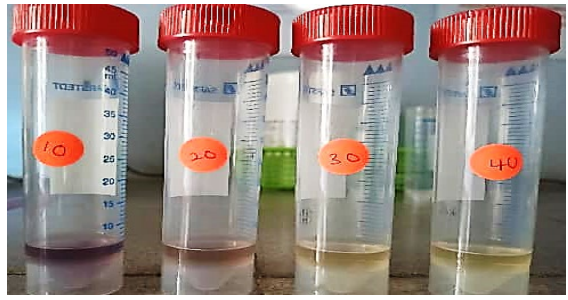

Figure 4: Ascorbic acid standards after adding DPPH solution.

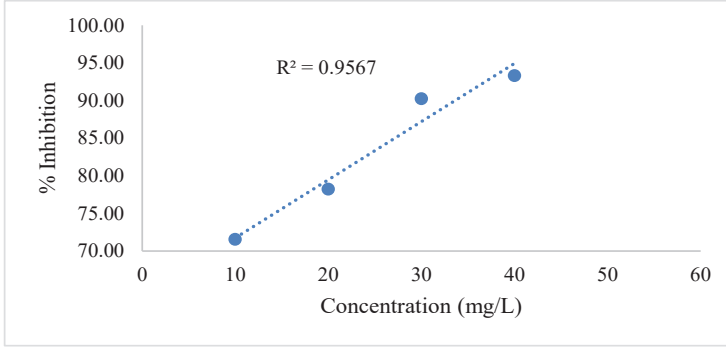

Figure 5: Standard curve of Ascorbic Acid.

For the sample, five concentrations were made starting from 10 ppm to 50 ppm with increments of 10 ppm. The violet colour in the sample mixture remained and did not change to yellow colour but instead to pale purple (shown in Figure 6).

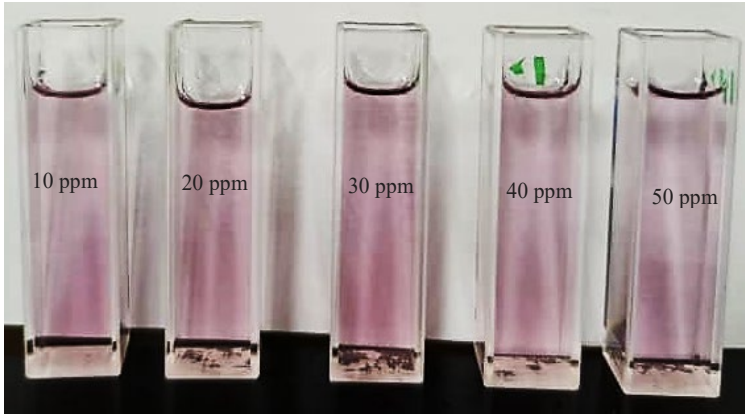

Figure 6: Sample after adding DPPH solution.

After comparison with the standard, it was found that the standard colour with an absorbance of around 0.2 also appears in purple with low intensity. However, the absorbance showed that the samples have a good capability to scavenge free radicals from DPPH which ranges from 0.16 to 0.24. The graph that has been plotted below (Figures 7 and 8) is the best-fit line consisting of only four concentrations excluding the 20 ppm concentration. This was done for both samples (day 7 and day 17) and the concentrations chosen were also the same. In a DPPH assay, a higher sample concentration leading to lower absorbance indicates stronger antioxidant activity. The lower concentration of a sample needed to achieve 50% inhibition of DPPH radicals (lower IC_{50}), confirms its stronger antioxidant activity. This has been proven in this study as shown in Table 4.

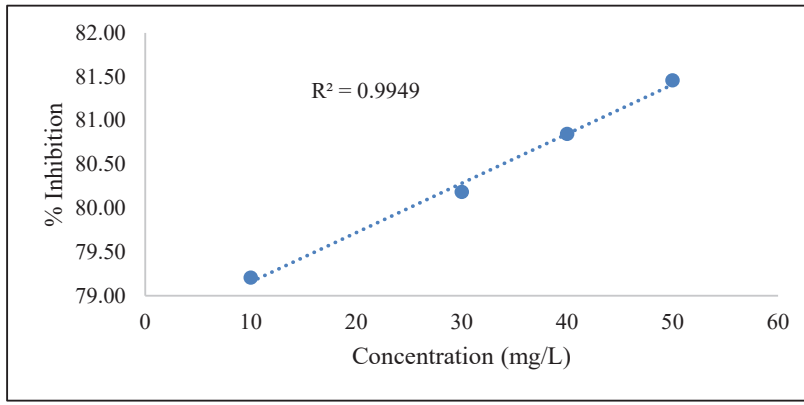

Figure 7: DPPH sample (day 7).

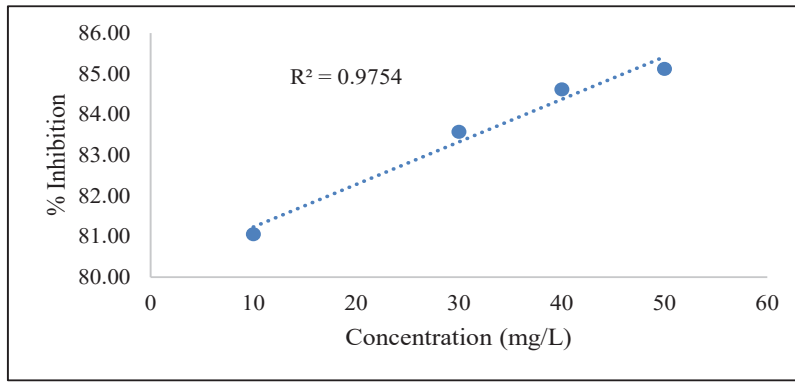

Figure 8: DPPH sample (day 17).

Kombucha fermentation significantly amplifies its antioxidant potential. The increase in antioxidants throughout the fermentation process is due to two main processes. Firstly, the microbial transformation of flavanols and other flavonoids into conjugated structures with free 3-OH bonds drastically increases their antioxidant capacity. Secondly, the synthesis of vitamin C, a potent antioxidant, is catalyzed by specific bacteria like *Gluconobacter* during glucose metabolism. In addition, based on Barakat [11], the concentration of water-soluble vitamins in kombucha is 0.74 mg/mL for vitamin B1, 0.52 mg/mL for vitamin B6, 0.84 mg/mL for vitamin B12, and 1.51 mg/mL for vitamin C. As kombucha ferments, there is

a notable escalation in the concentration of vitamin C and the overall antioxidant activity, resulting in a beverage with enhanced protective properties against oxidative stress. Not only that but according to Barakat [11], enzyme degradation of complex polyphenols into smaller molecules can indeed lead to an increase in the total phenolic compound measurement. This is because many analytical methods used to determine TPC are based on colorimetric reactions that respond to the presence of phenolic groups. By breaking down larger and more complex polyphenols into smaller molecules with multiple phenolic groups, the number of reactive sites for these assays increases.

Table 4: DPPH assay result for standard and sample.

Standard/ Sample	Concentration (mg/L)	Absorbance (at 513 nm)	R^2	Inhibition (%)	IC_{50} (mg/L)
Standard	10	0.3218	0.9567	71.57	23.0371
	20	0.2465		78.22	
	30	0.1103		90.26	
	40	0.0756		93.32	
Sample (day 7)	10	0.2354	0.9949	79.20	46.2779
	30	0.2243		80.19	
	40	0.2168		80.85	
	50	0.2099		81.46	
Sample (day 17)	10	0.2145	0.9754	81.05	28.0178
	30	0.1860		83.57	
	40	0.1742		84.61	
	50	0.1685		85.11	

Total Phenolic Content (TPC)

TPC is one of the important parameters of total antioxidant capacity (TAC) and is widely used for the evaluation of the antioxidant properties of plants. Gallic acid has been used as a standard for this test. Five different concentrations of gallic acid (10 ppm, 50 ppm, 70 ppm, 100 ppm) have been prepared. Blank containing all reagents except gallic acid was used. To determine the TPC in the sample, a calibration curve was constructed using gallic acid calibration standards. The coefficient of determination

(R^2) of the resulting calibration curve (y = 0.0051x - 0.0007) was 0.9994, suggesting excellent linearity in the studied range of concentrations (shown in Figure 9). The wavelength used was 760 nm.

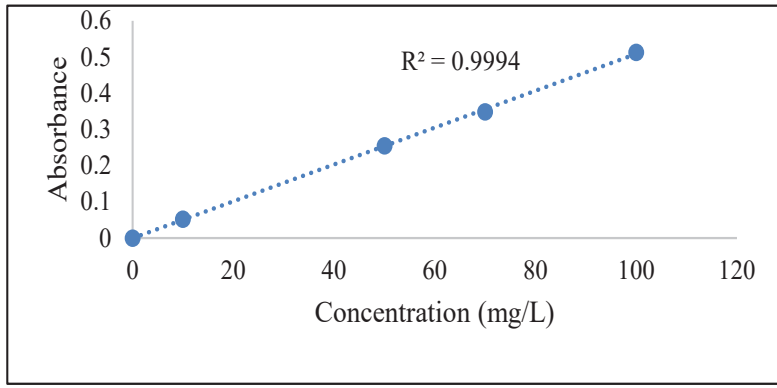

Figure 9: Standard curve of Gallic Acid.

1000 ppm of stock solution has been used for both days of fermentation. For blank, distilled water has been used to replace the sample. A light blue colour appeared after adding sodium carbonate (as shown in Figure 10). When a sample, suspected of containing phenolic compounds is combined with the Folin-Ciocalteu reagent under alkaline conditions, a chemical interaction occurs. This reaction manifests itself visually as the formation of a blue-coloured solution. Importantly, the intensity of this blue staining is directly proportional to the concentration of phenolics present in the sample.

Figure 10: Blank and sample for TPC test.

Analysis of the total polyphenol content in kombucha revealed that the content of these compounds belonging to black tea increases as fermentation time progresses. The polyphenol content observed on the 17th day of fermentation was higher than on the 7th day of fermentation (as shown in Table 5).

Table 5: TPC result for sample.

Day of fermentation	Absorbance (at 760 nm)	TPC (mg GAE/mL)	TPC (mg GAE/L)
7	0.0069	1.4902	1490.1961
17	0.0116	2.4118	2411.7647

Total Flavonoid Content (TFC)

TFC was calculated from a calibration curve based on a reference flavonoid standard measured at the same experimental conditions and wavelength. For tea samples, quercetin has been reported to be a suitable standard for determining TFC [22]. TFC content is usually determined using a colorimetric $AlCl_3$ test without or with $NaNO_2$ (nitrating agent) at an absorption wavelength between 400 and 550 nm depending on the reagent used [24]. In this study, the wavelength that has been used is 510 nm which is in the range stated. According to Nguyen *et al.* [22], a complex formed with $NaNO_2$ in an alkaline medium for catechins makes it suitable for tea and has a significant absorption at 510 nm.

Four different concentrations of quercetin (15 ppm, 50 ppm, 100 ppm, 200 ppm) have been prepared. Blank containing all reagents except quercetin was used (Figure 11). To determine the TFC in the sample, a calibration curve was constructed using quercetin calibration standards (shown in Figure 12). 1000 ppm of stock solution has been used for both days of fermentation. For blank, distilled water has been used to replace the sample. The linear regression equation for quercetin was derived as $y = 0.0015x + 0.0109$ and the R^2 value obtained was 0.9859.

Figure 11: Blank and sample for TFC test.

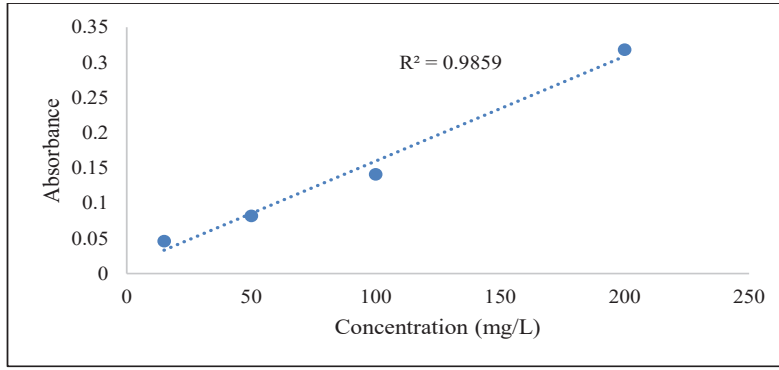

Figure 12: Standard curve of Quercetin.

The resulting complexes cause a colour change, and the extent of this change is used as a proxy for the total flavonoid content present in the kombucha sample. $AlCl_3$ acts like a magnet for flavonoids in kombucha, causing a colour change (usually yellow to orange). The stronger colour indicates more flavonoids present. Analysis of the total flavonoid content in kombucha revealed that the content of these compounds belonging to black tea increases as fermentation time progresses. The polyphenol content observed on the 17th day of fermentation was higher than on the 7th day of fermentation (as shown in Table 6).

Table 6: TFC result for sample.

Day of fermentation	Absorbance (at 510 nm)	TFC (mg QE/mL)	TFC (mg QE/L)
7	0.0110	0.0667	66.6667
17	0.0118	0.6000	600.0000

Development of Kombucha Serum Stick

The development of kombucha stick serum begins with the strategic selection of kombucha fermentation stages. From the antioxidant tests that have been done, kombucha liquid harvested on the 17th day of fermentation was selected for use. This time frame was chosen to maximize the potential concentration of beneficial antioxidant compounds in the final product. The most suitable formulation that has been explored was formulation 2 due to its miscibility characteristics. Table 7 shows the impact of kombucha-based formulation (F2 and F3) on the pH of the final product compared to a control. While the starting kombucha liquid had a naturally acidic pH of 2.135, directly applying it would not be ideal for facial skin because of high acidity. Fortunately, the formulation process successfully adjusts the pH. This demonstrates the effectiveness of the chosen formulation in creating a kombucha-based product suitable for topical application on facial skin without disrupting its natural acidic balance.

Table 7: pH measurement.

Formulation	pH	
	Before (kombucha liquid)	After (product)
Control	-	4.975
F2	2.135	4.721
F3	2.135	4.588

Patch testing involves applying a small amount of the product to the skin and leaving it on to see if a reaction develops. To evaluate the potential for irritation or sensitization, a human patch test was conducted following established protocols. Ten participants were recruited for this study. A small quantity of the kombucha-based serum sticks formulations (F2 and F3), along with a control substance, was applied to designated areas on their skin. These patches were left undisturbed for approximately 30 minutes to allow for potential reactions to develop. The participants were then monitored

for any adverse effects over seven days, with observations recorded at designated intervals (day 1, day 3, day 5, day 7). Table 8 presents the results of human patch tests (where the χ symbol refers to no bad or adverse effects on the skin). The table evaluates potential negative reactions such as erythema (redness), pruritus (itching), burning sensation, increased surface roughness, and stinging. Notably, none of the participants exhibited any adverse reactions to either formulation (F2 or F3) or the control throughout the seven-day monitoring period. This absence of negative reactions suggests a favourable initial indication of the kombucha serum stick's potential for broad skin type compatibility.

Table 8: Kombucha serum stick effect on the skin.

Formulation	Effects on the skin				
	Redness	Itchy	Hot / Burn	Rough	Sting
Control	X	X	X	X	X
F2	X	X	X	X	X
F3	X	X	X	X	X

An organoleptic test was conducted to assess the sensory characteristics of the final product. Tables 9 and 10 summarize the results for appearance, colour, odour, smoothness, miscibility, and ease of use of the kombucha serum stick formulations (F2 and F3) compared to the control.

Table 9: Kombucha serum stick appearance.

Formulation	Appearance
Control (F1)	
F2	
F3	

Table 10: Kombucha serum stick organoleptic test.

Formulation	Colour	Miscibility	Odour	Smoothness	Ease of use
Control (F1)	Milky white	High	Strong: VCO + lavender	Very high	Easy
F2	Light ivory	High	Strong: VCO + lavender	High	Easy
F3	Dark ivory	Low	Strong: Kombucha sensation Mild: VCO + lavender	Very low	Hard

Visually, only the control exhibited a milky white appearance, while for formulations 2 and 3, it formed an ivory color with different intensity. The chosen lavender and Virgin Coconut Oil (VCO) fragrance successfully permeated all samples, resulting in a strong scent. However, formulation 3 (F3) proved challenging to mix, resulting in a product with separated oil and water phases, indicating an unsuccessful combination of ingredients. Despite initial blending, the product exhibited phase separation over time, with the kombucha's water content migrating to the surface. Olivem 1000, a potent emulsifier for skincare, was employed to improve the mix-ability of the formulation. However, despite its use, phase separation persisted. This could be attributed to an unsuitable water-to-oil ratio, particularly the 1:1 kombucha liquid-to-beeswax ratio. This could also be due to the insufficient amount of Olivem 1000 for F3, which may not be enough to emulsify the water and oil. The control group received high marks for smoothness, followed by formulation 2 (F2), signifying a well-developed product with a desirable texture for topical application. Formulation 3 (F3) received a very low smoothness rating, indicating a textural concern. In terms of ease of use, the control formulation received a positive rating. Formulation 2 was considered easy to use, but formulation 3 was found to be difficult to apply. Overall, the organoleptic test results demonstrate consistent visual properties and fragrance delivery across all formulations, except for formulation 3. The serum sticks remained intact at room temperature, exhibiting no melting or deformation, except for formulation 3, which experienced liquid discharge.

F3 also suffered from a rough texture that required excessive blending time. In comparison, F2 demonstrated superior smoothness and holds significant promise for future serum stick development.

CONCLUSION

In retrospect, healthy kombucha (black tea) fermented tea was successfully prepared. Olfactory evaluation reveals a progressive transformation in the aroma, indicating past fermentation. Visually, a new translucent pellicle, indicating SCOBY growth, appeared on the solution's surface. Documented pH levels showed a decrease from day 7 to day 17, a key indicator of successful fermentation. The colour of kombucha also changes significantly, from dark to light as fermentation progresses, indicating an increase in polyphenols. Following a thorough evaluation, the fermentation was stopped at two different points, day 7 and day 17. At each of these junctions, a small amount of kombucha was aseptically separated from the bulk solution. This isolated kombucha (stopping fermentation) was used for further analysis and serum stick manufacturing. This study also investigated fermentation duration's influence on black tea kombucha's antioxidant capacity. Three established methods were employed for quantitative assessment which are 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). The DPPH assay yielded IC_{50} values of 46.2779 mg/L and 28.0178 mg/L for kombucha fermented for 7 and 17 days, respectively. A lower IC_{50} value signifies a more potent antioxidant effect, indicating that kombucha from day 17 exhibited a statistically significant ($p < 0.05$) increase in its free radical scavenging ability. TPC and TFC analyses corroborated this trend. Both TPC and TFC demonstrated a positive correlation with fermentation duration. Kombucha fermented for 7 days exhibited a TPC of 1490.1961 mg GAE/L and a TFC of 66.6667 mg QE/L. Notably, these values were significantly lower compared to day 17 kombucha, which displayed a TPC of 2411.7647 mg GAE/L and a TFC of 600 mg QE/L. Samples from day 17 were utilized to produce the final product. A critical aspect of the development process involved ensuring the stability and safety of the serum in stick format. The absence of fungal growth on serum is also positive feedback. Furthermore, a comprehensive evaluation of the physical properties of the formulation was carried out. This evaluation includes patch testing on human subjects to

assess potential skin irritation and allergic reactions. In addition, a sensory analysis (organoleptic test) is performed to evaluate factors such as texture, colour, and fragrance of the serum when used. The results of the safety assessment are encouraging. Based on visual observations during testing and in vivo results from patch testing, no adverse effects or potential harm to users were identified. These preliminary evaluations pave the way for further investigation into the efficacy of kombucha serum sticks to address targeted skin concerns.

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