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Fermentation Characteristics and Bioactive Properties of Kombucha *Senna alexandrina* Mill. Leaves

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Abstract

Kombucha is a fermented beverage produced by a symbiotic culture of bacteria and yeast (SCOBY) and is widely recognized for its functional properties. This study investigated the fermentation characteristics, bioactive properties, antibacterial activity, ethanol content, and sensory attributes of kombucha produced from senna leaves (*Senna alexandrina* Mill.) during different fermentation periods (0, 4, 8, and 12 days). Physicochemical parameters including pH, optical density, SCOBY biomass, reducing sugar, and total titratable acidity were monitored, while bioactive properties were evaluated through total phenolic content and antioxidant activity (IC_{50}). Antibacterial activity against *Escherichia coli*, ethanol content, and sensory characteristics were also analyzed. The results showed that fermentation significantly decreased pH while increasing total titratable acidity, optical density, and SCOBY biomass, indicating active microbial metabolism. Total phenolic content increased during fermentation from 96.80 to 119.56 µg GAE/mL, accompanied by a substantial improvement in antioxidant activity as indicated by decreasing IC_{50} values. Antibacterial activity against *E. coli* was absent in the unfermented sample but emerged after fermentation. Ethanol concentration increased gradually but remained very low (0.0141% at day 12), well below regulatory limits for non-alcoholic and halal beverages. Sensory evaluation showed that fermentation time influenced taste and aroma, while color remained relatively stable. Overall, the findings demonstrate that senna leaves can serve as a suitable herbal substrate for kombucha fermentation and contribute to the development of functional fermented beverages with enhanced bioactive properties.

Keywords: Antioxidant activity; Antibacterial activity; Kombucha fermentation; *Senna alexandrina*; Sensory evaluation.

INTRODUCTION

Kombucha is a fermented beverage traditionally produced from sweetened tea using a symbiotic culture of bacteria and yeast (SCOBY) (Onsun et al., 2025). During kombucha fermentation, microorganisms metabolize sugars into various organic acids, ethanol, vitamins, and other bioactive compounds that contribute to the beverage's functional properties (Andrade et al., 2025). Due to its potential health benefits, including antioxidant, antimicrobial, and probiotic effects, kombucha has gained increasing attention as a functional beverage worldwide (de Oliveira et al., 2023). Traditionally, kombucha is produced using black or green tea (*Camellia sinensis*). However, recent studies have explored the use of various herbal plants as alternative substrates in order to enhance the functional properties and diversify the sensory characteristics of kombucha. Herbal substrates may provide additional bioactive compounds such as polyphenols, flavonoids, and other phytochemicals that could improve the nutritional and therapeutic value of the kombucha beverage (Barakat et al., 2023).

One herbal plant with promising potential for kombucha production is *Senna alexandrina* Mill. (syn. *Cassia angustifolia* Vahl.), commonly known as senna or "jati cina". This medicinal plant has long been used in traditional medicine for its laxative, anti-inflammatory, anti-obesity, and cholesterol-lowering properties (Aronson, 2016; Thaker et al., 2023). The leaves of senna contain various bioactive compounds including flavonoids, tannins, alkaloids, and phenolic compounds, which are known to exhibit strong antioxidant activity (Ahmad et al., 2025). These compounds may contribute to the functional properties of fermented beverages produced from this plant.

Fermentation plays a critical role in determining the chemical composition and quality of kombucha (Wang et al., 2022). During fermentation, microbial metabolism leads to significant changes in physicochemical parameters such as pH, total acidity, reducing sugar content, and alcohol concentration (Aji, 2026). At the same time, microbial growth and metabolic activity may influence the formation of bioactive compounds and antioxidant capacity. Furthermore, the duration of fermentation can also affect sensory characteristics, including taste, aroma, and color, which are important factors influencing consumer acceptance (Dartora et al., 2023).

Despite the growing interest in herbal-based kombucha, the utilization of senna leaves as a substrate for kombucha fermentation has not been widely reported. Considering the rich phytochemical content and medicinal potential of this plant, further investigation is needed to understand its suitability as a substrate for kombucha production. Therefore, this study aims to evaluate the physicochemical characteristics, antioxidant activity, and sensory properties of kombucha produced from senna leaves during different fermentation periods. The findings of this study are expected to provide scientific insights into the potential of senna as a novel herbal substrate for the development of functional kombucha beverages.

MATERIALS AND METHODS

Preparation of Senna Leaves Kombucha

Dried senna leaves were used as the substrate for kombucha fermentation. A total of 30 g of dried senna leaves were added to 1500 mL of boiling distilled water and allowed to steep until the solution cooled to room temperature. The infusion was then filtered and supplemented with 150 g of sucrose as a carbon source. After the sugar was completely dissolved, the solution was transferred into sterile glass fermentation jars. A symbiotic culture of bacteria and yeast (SCOBY) was then inoculated into the prepared substrate as the fermentation starter. The fermentation containers were covered with sterile cloth and kept at room temperature under static conditions. Fermentation was conducted for 0, 4, 8, and 12 days. Each treatment was performed in triplicate using a completely randomized design.

Measurement of pH

The pH value of the kombucha samples was measured using a digital pH meter. Approximately 30 mL of kombucha sample was placed in a beaker, and the electrode of the pH meter was immersed into the sample until the reading stabilized. Measurements were carried out in triplicate for each fermentation period.

Microbial Growth Analysis

Optical Density (OD)

Microbial growth during fermentation was evaluated by measuring the optical density of the kombucha samples. A 2 mL aliquot of the kombucha sample was placed in a cuvette, and the absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 600 nm with distilled water as the blank. Measurements were conducted in triplicate.

Dry Weight of SCOBY

The SCOBY biomass produced during fermentation was measured as dry weight. The SCOBY formed at fermentation days 4, 8, and 12 was collected and weighed using an analytical balance. The samples were then dried in an oven at 85°C for 1.5 hours until a constant weight was obtained. The dry weight was recorded to determine biomass accumulation during fermentation.

Determination of Reducing Sugar Content

The reducing sugar content of kombucha was determined using the Nelson–Somogyi method with spectrophotometric analysis. A glucose standard solution was prepared and used to generate a calibration curve. For the analysis, 1 mL of kombucha sample was mixed with 1 mL of Nelson reagent and heated in a water bath for 30 minutes. After cooling to room temperature, 1 mL of arsenomolybdate reagent was added, followed by the addition of 7 mL of distilled water. The absorbance of the solution was measured using a UV–Vis spectrophotometer at a wavelength of 768 nm. The concentration of reducing sugar was calculated based on the glucose standard calibration curve.

Determination of Total Titratable Acidity

Total titratable acidity was determined using an acid–base titration method. Ten grams of kombucha sample were diluted with distilled water to a final volume of 250 mL in a volumetric flask. A 50 mL aliquot of the diluted sample was transferred into an Erlenmeyer flask and mixed with 3–4 drops of 1% phenolphthalein indicator. The solution was titrated with 0.1 N NaOH until a persistent pale pink color appeared. The total titratable acidity was calculated based on the volume of NaOH used during titration.

Determination of Total Phenolic Content

The total phenolic content of kombucha samples was determined using the Folin–Ciocalteu method. Kombucha samples were mixed with Folin–Ciocalteu reagent and sodium carbonate solution, followed by incubation under controlled conditions. The absorbance of the resulting solution was measured using a UV–Vis spectrophotometer, and the total phenolic content was expressed as gallic acid equivalents (GAE) based on the calibration curve prepared using gallic acid standards.

Antioxidant Activity Assay

The antioxidant activity of kombucha samples was evaluated using the DPPH radical scavenging assay. A solution of DPPH was prepared and mixed with kombucha samples at different concentrations. The mixture was incubated in the dark for a specified period, and the absorbance was measured using a UV–Vis spectrophotometer. The percentage of radical scavenging activity was calculated, and the IC_{50} value was determined as the concentration required to inhibit 50% of the DPPH radicals.

Antibacterial Activity Assay

The antibacterial activity of kombucha produced from senna leaves was evaluated using the agar well diffusion method against *Escherichia coli*. The bacterial cultures were first grown in nutrient broth and incubated at 37 °C for 24 h. The bacterial suspension was then adjusted to the turbidity of 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Subsequently, 100 µL of the standardized bacterial suspension was evenly spread onto sterile nutrient agar plates using a sterile cotton swab. Wells with a diameter of approximately 6 mm were created in the agar using a sterile cork borer, and 50 µL of kombucha sample was added into each well. Sterile distilled water was used as a negative control, while chloramphenicol (30 µg/mL) was used as a positive control. The plates were incubated at 37 °C for 24 h**ours**. Antibacterial activity was determined by measuring the diameter of the clear inhibition zone surrounding each well. The results were expressed as the mean inhibition zone diameter (mm) obtained from three independent replicates.

Ethanol Content Analysis

The ethanol content of kombucha samples was analyzed using gas chromatography (GC-FID). One milliliter of homogenized kombucha sample was transferred into a microtube and mixed with 1 mL of dichloromethane as the extraction solvent. The mixture was vortexed and centrifuged if necessary to obtain a clear solution. The clear extract was transferred into a GC vial, and 1 µL of the sample was injected into the gas chromatograph. Ethanol standards with different concentrations were used for calibration, and the ethanol concentration in the samples was determined based on the resulting chromatographic peaks.

Sensory Evaluation

Sensory evaluation was conducted using a hedonic test involving 30 untrained panelists. The kombucha samples obtained from different fermentation periods were evaluated based on three sensory attributes: color, aroma, and taste. Panelists were asked to rate their level of preference using a five-point hedonic scale, where 1 represented "strongly dislike" and 5 represented "strongly like." This sensory evaluation protocol involving human participants was approved by the Research Ethics Committee of Universitas Ahmad Dahlan, Yogyakarta, Indonesia (Ethical Approval No. 012405098).

Statistical Analysis

All experiments were conducted in triplicate using a completely randomized design. Data obtained from physicochemical analyses were presented as mean values. Sensory data were analyzed using normality and homogeneity tests. If the data were normally distributed, analysis of variance (ANOVA) followed by Tukey HSD was performed. If the data were not normally distributed, the Kruskal–Wallis test followed by Dunn's test was applied to determine significant differences among treatments.

RESULTS AND DISCUSSION

Physicochemical Changes during Fermentation

The physicochemical properties of kombucha produced from senna leaves during fermentation are presented in Table 1. The pH decreased significantly ($p < 0.05$) from 3.75 ± 0.03 at day 0 to 3.31 ± 0.03 at day 12. In contrast, the optical density (OD600) increased during fermentation, reaching 0.12 ± 0.02 at day 12, indicating increased microbial growth. The SCOBY dry weight also increased significantly ($p < 0.05$), from 0.00 ± 0.00 g at day 0 to 0.68 ± 0.04 g at day 12. Meanwhile, the reducing sugar content remained relatively stable throughout fermentation, ranging from 3.89 ± 0.11 mg/mL to 3.81 ± 0.04 mg/mL. Total titratable acidity increased markedly during fermentation, from 4.30 ± 0.18% at day 0 to 24.45 ± 0.31% at day 12, with significant differences observed among fermentation times ($p < 0.05$).

Table 1. Physicochemical changes in senna leaf kombucha during fermentation.

Fermentation Time (days)	pH	Optical Density (OD_{600})	SCOBY Dry Weight (g)	Reducing Sugar Content (mg/mL)	Total Titratable Acidity (%)
0	3.75 ± 0.03^a	0.08 ± 0.00^a	0.00 ± 0.00^c	3.89 ± 0.11	4.30 ± 0.18^d
4	3.44 ± 0.01^b	0.09 ± 0.03^a	0.50 ± 0.06^b	3.88 ± 0.17	8.30 ± 0.18^c
8	3.35 ± 0.01^c	0.09 ± 0.01^b	0.67 ± 0.09^a	3.88 ± 0.06	10.48 ± 0.25^b
12	3.31 ± 0.03^c	0.12 ± 0.02^b	0.68 ± 0.04^a	3.81 ± 0.04	24.45 ± 0.31^a

Data are expressed as mean ± standard deviation (n = 3). Means followed by different superscript letters within the same column indicate significant differences according to Tukey's HSD test at $p < 0.05$.

Bioactive Properties and Antibacterial Activity

The bioactive properties and antibacterial activity of kombucha produced from senna leaves during fermentation are presented in Table 2. The total phenolic content increased from 96.80 ± 3.85 µg GAE/mL at day 0 to 119.56 ± 8.18 µg GAE/mL at day 12, indicating a gradual accumulation of phenolic compounds during fermentation. Correspondingly, antioxidant activity improved as shown by the decreasing IC_{50} values from 74.19 ± 0.21 µg/mL to 20.56 ± 0.11 µg/mL, with significant differences among fermentation times ($p < 0.05$). Lower IC_{50} values indicate stronger antioxidant activity. Antibacterial activity against Escherichia coli was not detected at day 0 but appeared after fermentation. The largest inhibition zone was observed at day 4 (1.26 ± 0.08 mm), followed by day 8 (1.05 ± 0.12 mm) and day 12 (0.99 ± 0.19 mm). The inhibition zones produced by kombucha samples are shown in Figure 1. These results indicate that fermentation contributed to the emergence of antibacterial activity in the kombucha samples.

Table 2. Bioactive properties and antibacterial activity of kombucha produced from senna leaves during fermentation.

Fermentation Time (days)	Total Phenolic Content (µg GAE/mL)	Antioxidant Activity (IC_{50}, µg/mL)	Antibacterial Activity against *E. coli* (mm)
0	96.80 ± 3.85^{a}	74.19 ± 0.21^{d}	0.00 ± 0.00^{b}
4	102.58 ± 6.92^{a}	58.49 ± 0.15^{c}	1.26 ± 0.08^{a}
8	105.41 ± 6.35^{a}	43.99 ± 0.07^{b}	1.05 ± 0.12^{ab}
12	119.56 ± 8.18^{a}	20.56 ± 0.11^{a}	0.99 ± 0.19^{ab}

Data are expressed as mean ± standard deviation (n = 3). Means followed by different superscript letters within the same column indicate significant differences according to Tukey's HSD test at $p < 0.05$.

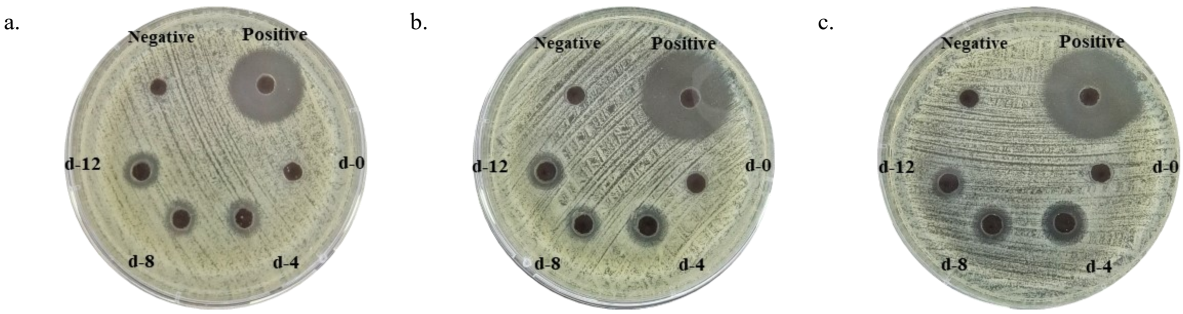

Figure 1. Antibacterial activity of kombucha produced from senna leaves against Escherichia coli using the agar well diffusion method.

Ethanol Content and Sensory Evaluation

The ethanol content and sensory evaluation of senna leaf kombucha during fermentation are presented in Table 3. Ethanol was not detected at day 0 but gradually increased during fermentation, reaching 0.0141% at day 12, indicating the formation of ethanol as a by-product of microbial metabolism. The sensory evaluation of senna leaf kombucha during fermentation is presented in Table 4. Color scores remained constant at 4.00 ± 0.00 throughout the fermentation period and showed no significant differences among treatments ($p > 0.05$). In contrast, taste and aroma scores showed significant differences across fermentation times ($p < 0.05$), with noticeable variations in panelist preference during the later stages of fermentation. These results indicate that fermentation time influenced panelists' perception of taste and aroma, while the color of the beverage remained relatively stable.

Table 3. Ethanol content and sensory evaluation of senna leaf kombucha during fermentation

Fermentation Time (days)	Ethanol (%)
0	ND
4	0.0033
8	0.0095
12	0.0141

Ethanol content was determined using GC-FID. ND indicates not detected.

Table 4. Ethanol content and sensory evaluation of senna leaf kombucha during fermentation.

Fermentation Time (days)	Sensory evaluation		
	Color	Taste	Aroma
0	4.00 ± 0.00[a]	4.50 ± 0.50[a]	3.83 ± 0.29[a]
4	4.00 ± 0.00[a]	3.33 ± 0.58[b]	3.00 ± 0.00[b]
8	4.00 ± 0.00[a]	4.33 ± 0.58[a]	3.83 ± 0.29[a]
12	4.00 ± 0.00[a]	3.00 ± 1.00[b]	2.00 ± 0.00[c]

Data are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$). Sensory attributes (color, taste, and aroma) were evaluated using a five-point hedonic scale by panelists.

Discussion

The results of this study demonstrate that fermentation significantly influenced the physicochemical properties, bioactive compounds, and functional characteristics of kombucha produced from senna leaves, highlighting the role of microbial metabolism in shaping the quality of the fermented beverage. The observed changes in pH, acidity, and SCOBY biomass during fermentation reflect the dynamic metabolic interactions within the microbial consortium of kombucha. The gradual decrease in pH throughout the fermentation period is primarily associated with the production of organic acids, particularly acetic and gluconic acids, generated by acetic acid bacteria as they oxidize ethanol produced by yeasts (Alves et al., 2025). This process contributes to the acidification of the fermentation medium, which is a characteristic feature of kombucha fermentation (Chong et al., 2024). Correspondingly, the substantial increase in total titratable acidity indicates intensified microbial metabolic activity and the accumulation of acidic metabolites over time. In parallel, the increase in optical density and SCOBY dry weight suggests active microbial proliferation and biofilm formation during fermentation. The SCOBY structure, composed of cellulose produced mainly by acetic acid bacteria, serves as a matrix that supports the coexistence and metabolic cooperation between yeasts and bacteria (Santiago-Santiago et al., 2025). Such microbial interactions facilitate substrate conversion and metabolite formation during fermentation. Similar fermentation patterns, characterized by decreasing pH, increasing acidity, and progressive SCOBY biomass formation, have been widely reported in kombucha produced from traditional substrates such as *Camellia sinensis*, as well as in various herbal-based kombucha beverages (Jakubczyk et al., 2020; Zheng et al., 2024). These findings indicate that senna leaves can effectively support typical kombucha fermentation kinetics and microbial growth dynamics.

The increase in total phenolic content observed during fermentation suggests that microbial activity within the SCOBY contributed to the transformation and release of phenolic compounds from the senna leaf substrate. This phenomenon is commonly associated with phenolic biotransformation, in which microbial enzymes such as β-glucosidase and esterase catalyze the hydrolysis of complex phenolic conjugates into simpler and more soluble forms (Alharbi, 2026). In addition, fermentation can promote the release of phenolic compounds bound to the plant or food matrix through the action of microbial enzymes, resulting in the formation of smaller free phenolics with improved bioavailability in the fermented beverage (Yang et al., 2023). Consistent with the results of this study, the increase in total phenolic content during fermentation suggests active phenolic biotransformation. The enhancement of antioxidant activity, indicated by the decrease in IC_{50} values, further supports the role of fermentation in improving the functional properties of kombucha. Lower IC_{50} values reflect a stronger ability of the sample to scavenge free radicals, which is often closely associated with increased phenolic content. This finding is consistent with previous studies showing that kombucha fermentation can enhance phenolic compounds and antioxidant capacity through microbial metabolism (Kim et al., 2023). In addition, the type of plant substrate used in kombucha production plays an important role in shaping the phenolic composition and antioxidant potential of the final beverage (Mihai et al., 2024).

The emergence of antibacterial activity against *Escherichia coli* after fermentation suggests that microbial metabolism during kombucha fermentation contributes to the production of antimicrobial compounds. This activity is likely associated with the accumulation of organic acids, particularly acetic acid, which lowers the environmental pH and creates unfavorable conditions for the growth of pathogenic bacteria (Aji et al., 2025). In addition, other fermentation-derived metabolites such as ethanol, phenolic compounds, and various secondary metabolites may also contribute to the inhibitory effect. Kombucha fermentation generates diverse bioactive compounds originating from both the substrate and microbial metabolism, many of which have been associated with antimicrobial activity (de Miranda et al., 2022). Kombucha fermentation significantly alters its chemical composition, generating diverse metabolites such as organic acids, alcohols, esters, and phenolic compounds that contribute to antimicrobial activity against pathogenic microorganisms (Al-Mohammadi et al., 2021). The transformation and enrichment of phenolic compounds during fermentation have also been reported to play an important role in the biological activity of kombucha, with more than 120 phenolic compounds identified that contribute to antioxidant and antibacterial properties (Cardoso et al., 2020). Previous studies have shown that kombucha beverages exhibit inhibitory activity against several pathogenic bacteria, including *E. coli*, and that this activity can be influenced by fermentation conditions such as substrate type, sugar concentration, and fermentation duration (Valiyan et al., 2021; Zubaidah et al., 2018). Recent research has further identified specific antibacterial compounds associated with the kombucha system, such as 5-hydroxymethylfurfural (HMF), which was isolated from SCOBY extracts and demonstrated strong inhibitory activity against several bacterial pathogens (Nam et al., 2025). Although the inhibition zones observed in this study were relatively small, the appearance of antibacterial activity after fermentation indicates that fermentation enhances the functional properties of the beverage by promoting the formation of bioactive antimicrobial metabolites.

The formation of ethanol during fermentation reflects the metabolic activity of yeasts present within the SCOBY consortium. During kombucha fermentation, sucrose is hydrolyzed into glucose and fructose, which are subsequently utilized by yeasts to produce ethanol and carbon dioxide through alcoholic fermentation (Sanwal et al., 2023). In kombucha systems, the ethanol produced by yeasts is subsequently oxidized by acetic acid bacteria into organic acids, particularly acetic acid, which contributes to the increasing acidity of the beverage. Previous studies have also reported that ethanol is a natural by-product of kombucha fermentation, with concentrations in commercial products typically ranging from nondetectable levels to several percent depending on fermentation conditions and microbial activity (Chan et al., 2021; Jang et al., 2021). Therefore, monitoring ethanol levels is an important aspect in evaluating the safety and regulatory compliance of kombucha beverages, particularly in relation to limits established for non-alcoholic drinks. Regulatory standards define non-alcoholic beverages as containing less than 0.5% alcohol by volume (ABV) in the United States and up to 1.1% ABV in Canada, while halal guidelines established by the Indonesian Council of Ulama (MUI) limit ethanol content in fermented beverages to a maximum of 0.5% ABV. Notably, the ethanol levels detected in this study remained well below the regulatory threshold for non-alcoholic beverages, indicating that senna leaf kombucha falls within acceptable limits for both non-alcoholic and halal beverage classification.

The sensory evaluation indicated that fermentation time influenced panelists' perception of taste and aroma, while the color of the beverage remained relatively stable throughout the fermentation process. The stability of color suggests that the visual appearance of the senna leaf kombucha was not substantially affected by fermentation under the conditions applied. In contrast, variations in taste and aroma are closely associated with metabolic transformations occurring within the SCOBY microbial consortium. The accumulation of organic acids during fermentation, particularly acetic acid, can increase acidity and sourness, which may influence the sensory perception and overall acceptability of the beverage at longer fermentation times (Aung & Eun, 2022). Consistent with the sensory results observed in this study, kombucha fermented for eight days exhibited a relatively balanced sensory profile compared with longer fermentation periods, suggesting that moderate fermentation may favor the development of desirable flavor characteristics before excessive acid accumulation occurs. In addition to organic acids, microbial metabolism during kombucha fermentation can generate various volatile compounds, including alcohols, aldehydes, esters, and terpenoid derivatives, which contribute to the characteristic aroma complexity of the beverage (Xu et al., 2024). Therefore, the interaction between acid accumulation and volatile metabolite formation plays a key role in shaping the sensory attributes of senna leaf kombucha during fermentation. These findings highlight senna leaves as a promising medicinal plant substrate capable of supporting kombucha fermentation while enhancing the functional properties of the beverage. Further studies involving metabolomic profiling, microbial community analysis, and toxicity assessment are required to better elucidate the bioactive potential and safety of senna leaf kombucha.

CONCLUSIONS

This study demonstrates that senna leaves can serve as a suitable substrate for kombucha fermentation, supporting typical fermentation dynamics characterized by decreasing pH, increasing acidity, and microbial biomass formation. Fermentation enhanced the functional properties of the beverage, as reflected by increased phenolic content, improved antioxidant activity, and the emergence of antibacterial activity against *Escherichia coli*. Ethanol concentrations remained very low throughout fermentation and were well below regulatory limits for non-alcoholic and halal beverages. Sensory evaluation indicated that fermentation time influenced taste and aroma, with moderate fermentation providing a more balanced sensory profile. These findings highlight the potential of *Senna alexandrina* as a alternative herbal substrate for producing functional kombucha beverages, although further studies involving metabolomic profiling, microbial community analysis, and safety evaluation are recommended to better understand its bioactive potential.

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