Journal of Food Health and Bioenvironmental Science (September – December 2026), 19(3), e269550
https://doi.org/10.65763/jfhb.2026.e269550

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/



Effects of SCOBY Fermentation on the Phytochemical Profile, Antioxidant and Antibacterial Activity of *Sonneratia caseolaris* (L.) Engl. Leaf Infusion

M. Rifqi Efendi[a]*, Faris Fadil Darmawan[a], Edo Septian Roza[a], Alfin Najaha Pratama[a], Mesa Sukmadani Rusdi[b], Yuliawati Yuliawati[a], Puspa Dwi Pratiwi[a], Novia Tri Astuti[a] & Vina Neldi[a]

[a] *Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, 36122 Indonesia*
[b] *Department of Pharmacy, Politeknik Kesehatan Kementerian Kesehatan Jambi, Jambi, 36128 Indonesia*

Article info

Article history:
Received: 17 October 2025
Revised: 11 March 2026
Accepted: 9 April 2026

Keywords:
Sonneratia caseolaris, Kombucha, Antioxidant, Antibacterialn

Abstract

Indonesia harbors extensive mangrove ecosystems with considerable untapped potential as sources of functional bioactive ingredients. *Sonneratia caseolaris* (L.) Engl., a mangrove species traditionally used in ethnomedicine, contains diverse polyphenolic compounds. However, its application as a functional beverage remains unexplored, particularly regarding how SCOBY fermentation may influence its phytochemical composition and biological activities. This study evaluated the effects of SCOBY fermentation on the physicochemical characteristics, phytochemical profile, antioxidant capacity, and antibacterial activity of *S. caseolaris* leaf infusion. Fermentation was conducted for 14 days, and a fermented product was compared with an unfermented sample. The fermentation process resulted in a reduction in pH. Phytochemical analysis showed an increase in total phenolic content (TPC) from 21.92±0.18 to 24.23±0.09 mg GAE/g (≈10.5%) and total flavonoid content (TFC) from 11.02±0.06 to 11.58±0.13 mg QE/g (≈5.1%). UPLC–MS profiling revealed fermentation-associated alterations in phytochemical composition, including changes in flavonoid glycosides and sterol derivatives. Antioxidant activity evaluated using the DPPH assay showed comparable IC_{50} values between the unfermented infusion (46.05±0.12 µg/mL) and its fermented counterpart (45.26±0.26 µg/mL). Notably, the unfermented infusion exhibited no antibacterial activity, whereas the fermented product demonstrated strong inhibition against *Pseudomonas aeruginosa* (16±2.00 mm at 5%) and weak inhibition against *Staphylococcus aureus* (7.33±1.15 mm at 5%). Overall, SCOBY fermentation modified the chemical profile and generated selective antibacterial activity. These findings support the potential of fermented *S. caseolaris* leaf infusion as a potential functional beverage and contribute to the valorization of mangrove plant resources.

* Corresponding Author
e-mail: mrifqi@unja.ac.id

Journal of Food Health and Bioenvironmental Science (September – December 2026), 19(3), e269550
https://doi.org/10.65763/jfhb.2026.e269550

Introduction

Mangrove ecosystems represent a unique reservoir of bioactive plant species adapted to extreme environmental conditions, such as high salinity, tidal fluctuation, and microbial pressure. These ecological stresses stimulate the production of diverse secondary metabolites, including phenolic acids, flavonoids, sterols, and anthraquinones, many of which exhibit antioxidant and antimicrobial properties (Abeysinghe, 2010; Bandaranayake, 2002; Kathiresan & Bingham, 2001).

Among Indonesian mangrove species, *Sonneratia caseolaris* (L.) Engl. has attracted attention due to its ethnomedicinal relevance and phytochemical richness. Previous phytochemical investigations have identified flavonoids such as luteolin, quercetin, vitexin derivatives, sterol glycosides, and anthraquinones in different parts of *S. caseolaris* (Halifah et al., 2019; Liu et al., 2023; Sadhu et al., 2006). Various plant parts have demonstrated pharmacological activities including antiseptic, analgesic, anti-inflammatory, antimicrobial, and antioxidant effects (Cerri & Galli, 2025; Kundu et al., 2023; Pagarra et al., 2022; Rao, 2020). Extracts from fruits, bark, and leaves have demonstrated antioxidant and antibacterial activities against pathogens, including *Staphylococcus aureus,* methicillin-resistant *S. aureus, Bacillus subtilis* and *Escherichia coli* (Audah et al., 2020, 2022; Simlai et al., 2014; Tran et al., 2023).

Ethnobotanical reports indicate that *S. caseolaris* leaves have been traditionally used in local medicinal preparations. Leaves and other plant parts are used for treating helminthiasis, coughs, hematuria, and smallpox, and are applied as poultices for wound treatment (Arung et al., 2015; Halifah et al., 2019; Kalor et al., 2025; Pagarra et al., 2022). Such traditional use suggests prior human exposure and suggests a preliminary indication of safety for aqueous preparations.

However, most previous investigations have focused on organic solvent extracts, while the functional potential of aqueous leaf infusion preparations remains limited. Leaves represent a renewable biomass resource compared with fruits, and aqueous infusion is consistent with traditional herbal preparation methods while avoiding organic solvent residues. Although *S. caseolaris* fruit has been more widely documented for food applications (Rao, 2020), the development of fermented leaf infusion as a functional beverage has not been previously reported.

Fermentation using a symbiotic culture of bacteria and yeast (SCOBY), commonly applied in kombucha production, has been shown to modify phytochemical composition and influence biological activity (Jayabalan et al., 2014). Microbial enzymatic systems active during fermentation may alter polyphenolic structures, potentially influencing antioxidant and antimicrobial properties, particularly in tannin-rich plant substrates (Antolak et al., 2021; Villarreal-Soto et al., 2018).

Several studies have reported altered phenolic accessibility and antimicrobial activity following fermentation of herbal materials (Barakat et al., 2022). Microbial enzymes produced during fermentation can transform complex polyphenolic compounds into smaller or more accessible molecules, potentially altering antioxidant and antimicrobial properties, particularly in tannin-rich plant substrates (Diez-Ozaeta & Astiazaran, 2022; Jayabalan et al., 2014). Similar effects have been observed in fermented plant beverages such as tea, where fermentation alters phenolic composition and bioactivity (Antolak et al., 2021). These findings suggest that fermentation may modify the bioactive profile of polyphenol-rich plant materials, including mangrove-derived species. Therefore, this study aimed to evaluate the effects of SCOBY fermentation on phytochemical profile, antioxidant capacity, and antibacterial activity of a *S. caseolaris* leaf infusion.

Materials and methods

1. Plant material

S. caseolaris leaves were collected from the mangrove area of Suak Labu Village, Kuala Betara Sub-district, Jambi Province, Indonesia. The plant was taxonomically identified, and a voucher specimen (No. 18/HB/05/2023) was deposited at the Jatinangor Herbarium. Only mature leaves were selected to reduce variability in phytochemical composition. The collected leaves were washed and dried in drying oven (UN30, Memmert GmbH + Co. KG, Germany) at 50°C for 3 days until a constant weight was achieved. The dried material was then ground into coarse powder prior to preparation of the leaf infusion.

2. Preparation of *S. caseolaris* leaf infusion and kombucha fermentation

Dried leaf powder (5 g) was steeped in 100 mL of distilled water at 70°C for 15 min to prepare the infusion (Joevanda et al., 2024). The resulting infusion was filtered using Whatman No. 1 filter paper, and the volume was adjusted to 100 mL with distilled water. After cooling to room temperature (25°C), 10 g of sucrose was added,

and the infusion was inoculated with 3 g (wet weight) of SCOBY. The SCOBY culture was obtained from a commercially available source, purchased through an online marketplace. Fermentation was carried out statically at 25–28°C for 14 days under aerobic conditions. No post-fermentation volume adjustment was performed prior to subsequent analyses.

3. Determination of total phenol content

The total phenolic content (TPC) of the *S. caseolaris* leaf infusion and the resulting kombucha was determined using the Folin-Ciocalteu spectrophotometric method, following the Indonesian Herbal Pharmacopoeia with minor adaptations (Ministry of Health Indonesia, 2017). Quantification was performed using a gallic acid calibration curve. Briefly, a stock solution (400 µg/mL) was prepared by dissolving 10 mg of gallic acid in 25 mL of ethanol. This solution was subsequently diluted with aquadest to generate a standard series with concentrations ranging from 25 to 60 µg/mL. In the assay, 1 mL aliquots of each standard or sample were mixed with 5 mL of diluted Folin-Ciocalteu reagent (7.5% v/v). The mixtures were vortexed and allowed to stand for 8 min at room temperature. Thereafter, 4 mL of a sodium carbonate solution (1% w/v) was added to alkalize the medium. After vigorous mixing, the reaction mixtures were incubated in the dark for 60 min to complete color development. The absorbance of the solutions was measured at 730 nm against a blank using a UV-Vis spectrophotometer (GENESYS 180, Thermo Fisher Scientific Inc., USA). The TPC of the samples was calculated by interpolation from the gallic acid standard curve and expressed as milligrams of gallic acid equivalents/g of dry simplicia (mg GAE/g).

4. Determination of total flavonoid content

The total flavonoid content (TFC) in both the *S. caseolaris* leaf infusion and the fermented kombucha was analyzed following the aluminum chloride colorimetric assay outlined in the Indonesian Herbal Pharmacopoeia, with specific adaptations (Ministry of Health Indonesia, 2017). A quercetin standard stock solution (400 µg/mL) was prepared in ethanol and subsequently diluted to create a calibration series with concentrations ranging from 3–75 µg/mL. The analytical procedure involved combining 1.0 mL of each standard or sample with 1.5 mL of ethanol. This was followed by the sequential addition of 0.1 mL of aluminum chloride solution (10% w/v) and 0.1 mL of sodium acetate solution (1 M). The mixture was then diluted to a total volume of 5 mL with 2.8 mL of distilled water and vortexed thoroughly. The reaction mixtures were incubated for 30 min at room temperature under light-protected conditions. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer (GENESYS 180, Thermo Fisher Scientific Inc., USA) against a prepared reagent blank. The total flavonoid content was determined through interpolation from the quercetin standard curve and reported as mg of Quercetin Equivalents/g of dry simplicia (mg QE/g).

5. Secondary metabolite profiling via UPLC-MS

Secondary metabolite profiling was performed using ultra-performance liquid chromatography coupled with mass spectrometry. UPLC-QTOF-MS/MS analysis was performed using an ACQUITY UPLC® H-Class System coupled with a Xevo G2-S QTOF mass spectrometer (Waters Corporation, USA). Separation was achieved using an HSS C18 column (100×2.1 mm, 1.8 µm; Waters Corporation, USA) maintained at 50°C. The mobile phase consisted of (A) 5 mM ammonium formate in water and (B) acetonitrile containing 0.05% formic acid, delivered at a flow rate of 0.2 mL/min using a step gradient over 23 min. Filtered samples (0.2 µm) were injected at a volume of 5 µL. Mass spectrometric analysis was performed using electrospray ionization (ESI) in positive ion mode with an m/z range of 50–1200. Source parameters were set as follows: source temperature, 100°C; desolvation temperature, 350°C; cone gas flow, 0 L/h; and desolvation gas flow, 793 L/h. A collision energy gradient of 4–60 eV was applied. Data acquisition and processing were carried out using MassLynx software (version 4.1, Waters Corporation, USA). Compound identification was considered tentative and was based on accurate mass measurement, molecular formula prediction, and comparison with reported literature data (Ismed et al., 2021).

6. Determination of antioxidant activity

The free radical scavenging potential of the samples was quantified through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition method, implemented with modifications from the protocol established by Rusdi et al. (2025). Sample solutions were prepared in methanol across a concentration gradient ranging from 1.56 to 100 µg/mL. For the test procedure, 0.2 mL aliquots of each diluted sample were reacted with 3.8 mL of a 50 µM methanolic DPPH solution. The resulting mixtures were homogenized and subsequently incubated under dark conditions for a 30-min period to facilitate the redox reaction. After the incubation phase, the absorbance of the solutions was recorded at 517 nm. A reference control

Journal of Food Health and Bioenvironmental Science (September – December 2026), 19(3), e269550
https://doi.org/10.65763/jfhb.2026.e269550

containing an equivalent volume of methanol in place of the sample was processed concurrently. The percentage of radical scavenging activity (% RSA) was determined using the following calculation:

$$\%\ \text{scavenging of DPPH} = [(A\ \text{control} - A\ \text{sample})/A\ \text{control}] \times 100$$

where A control (blank) represents the absorbance of the DPPH control reaction without a sample and A sample denotes the absorbance in the presence of the test sample. The half-maximal inhibitory concentration (IC_{50}) was derived by analyzing the concentration-response relationship. A linear regression model (y=a+bx) was fitted to the data points, where y represents the % RSA and x signify the sample concentration. The IC_{50} value was computed from the regression parameters using the formula:

$$IC_{50} = (50 - a)/b$$

7. Evaluation of antibacterial activity using disk diffusion method

The antibacterial properties were assessed following the standard disk diffusion protocol. Bacterial suspensions were first standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL) in sterile physiological saline (0.9% NaCl). A 100 µL aliquot of each adjusted suspension was then aseptically spread onto nutrient agar plates to generate uniform bacterial lawns. Sterile paper disks (6 mm in diameter) were impregnated with 10 µL of the following test solutions: *S. caseolaris* leaf infusion and kombucha fermentation products at concentrations of 5%, 2.5%, and 1.25% (b/v), a negative control (pure DMSO), and a positive control (chloramphenicol at 3 mg/mL in DMSO) against *S. aureus* ATCC 25923, *Propionibacterium acnes* ATCC 6919, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. The loaded disks were carefully placed onto the inoculated agar surfaces. All plates were subsequently incubated at 37°C for 24 h. After the incubation period, the diameters of the inhibition zones were measured in mm using a calibrated caliper. Each test condition was replicated 3 times to ensure result reliability (Efendi et al., 2024). The antibacterial activity was categorized according to established criteria (Harun et al., 2016) as follows: strong (>16 mm), moderate (11–15 mm), weak (6–10 mm), or no activity (<6 mm or absence of inhibition zone).

8. Data analysis

Data are presented as mean±standard deviation (SD) of triplicate measurements from three independent experiments. Statistical analyses were performed using SPSS software (version 24, IBM Corporation, USA). Normality was evaluated using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test prior to parametric analysis. All datasets met assumptions of normal distribution and homogeneity ($p>0.05$). Statistical analyses were performed using independent t-test for TPC and TFC, and one-way analysis of variance (ANOVA) with Duncan's post-hoc test for multiple comparisons for antioxidant evaluation. Significant differences among treatment groups were determined at $p<0.05$, with differing superscript letters indicating statistical significance.

Results and discussion

1. Effect of fermentation on pH of *S. caseolaris* leaf kombucha

The pH of the unfermented *S. caseolaris* leaf infusion was 4.0 and decreased to 3.0 after 14 days of SCOBY fermentation. This reduction reflects the production of organic acids during microbial metabolism of sucrose by the SCOBY. Kombucha fermentation is known to generate various organic acids, including acetic and gluconic acids, resulting in progressive acidification of the fermentation medium (Chen & Liu, 2000; Diez-Ozaeta & Astiazaran, 2022; Jayabalan et al., 2014). The observed decrease in pH is consistent with previous studies reporting acid accumulation as a characteristic outcome of kombucha fermentation (Antolak et al., 2021; Villarreal-Soto et al., 2018).

2. Fermentation-associated changes in phenolic and flavonoid content

Fermentation of *S. caseolaris* leaf infusion resulted in a statistically significant increase in total phenolic content (TPC), from 21.92±0.18 to 24.23±0.09 mg GAE/g ($p<0.05$), corresponding to an approximate 10.5% increase. Total flavonoid content (TFC) similarly increased from 11.02±0.06 to 11.58±0.13 mg QE/g ($p<0.05$), representing a 5.1% increase (Table 1). Although the increases were statistically significant, their relatively small magnitude suggests that fermentation modestly affected the overall phenolic and flavonoid levels rather than causing a substantial enhancement in phytochemical content. Such changes may reflect enhanced extractability of phenolic compounds or structural modification of glycosylated flavonoids during fermentation, as previously reported in kombucha-

fermented herbal substrates (Chen & Liu, 2000; Jayabalan et al., 2014).

Our findings are consistent with previous studies on the microbial fermentation of plant substrates. Leal et al. (2018) reported similar increases in phenolic accessibility during kombucha fermentation, attributing this effect to microbial enzymatic releases of bound phytochemicals. Likewise, Villarreal-Soto et al. (2018) demonstrated that kombucha fermentation enhances the availability of bioactive compounds through microbial bioconversion processes.

Table 1 Total phenolic and flavonoid contents and antioxidant activity (DPPH, IC_{50}) of *S. caseolaris* leaf infusion, fermented infusion, and gallic acid standard

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity (DPPH, IC50 µg/mL)
S. caseolaris leaf infusion	21.92±0.18^{b}	11.02±0.06^{b}	46.05±0.12^{a}
SCOBY-Fermented *S. caseolaris* leaf infusion	24.23±0.09^{a}	11.58±0.13^{a}	45.26±0.26^{a}
Gallic acid (standard)	–	–	38.28±1.6^{b}

Remark: Values are presented in mean±SD; values with different letters in the columns indicate statistically significant differences ($p<0.05$).

3. UPLC–MS profiling of phytochemical composition

UPLC–MS analysis was conducted to characterize the phytochemical composition of the *S. caseolaris* leaf infusion and its fermented kombucha counterpart. Representative chromatograms are shown in Fig. 1, while the tentative identification of detected metabolites is summarized in Table 2. Compound annotation was performed based on accurate mass measurements, characteristic fragmentation patterns, and comparison with previously reported literature data. The infusion contained a variety of bioactive constituents, predominantly phenolic compounds and flavonoids. Several compounds were tentatively identified, including mangiferin, epigallocatechin, rutin, luteolin-7-O-glucoside, quercitrin, apigenin, quercetin, and luteolin. These findings are consistent with previous phytochemical reports describing the presence of phenolic and flavonoid constituents in *S. caseolaris* leaves (Audah et al., 2020, 2022; Chen et al., 2015; Dahibhate et al., 2018; Liu et al., 2023; Sadhu et al., 2006).

Following fermentation, noticeable changes in the chromatographic profiles were observed, including variations in relative peak intensities and the appearance or reduction of several signals. For instance, a sterol-related signal (m/z 577.189) showed reduced relative intensity in the fermented sample, whereas a flavone glycoside signal (m/z 595.1663) became more pronounced after fermentation. Such alterations suggest that fermentation may influence the phytochemical matrix of the infusion through microbial metabolism or enzymatic biotransformation of polyphenolic compounds. These processes may involve hydrolysis of glycosidic bonds, oxidation–reduction reactions, or the release of bound phenolic constituents from plant matrices. In kombucha fermentation systems, the formation and transformation of bioactive compounds are driven by complex metabolic interactions among microorganisms within the SCOBY. During fermentation, microbial enzymes can modify plant-derived metabolites, leading to the production of structurally altered compounds or the enrichment of certain metabolites that were previously present at low concentrations (Jayabalan et al., 2014; Villarreal-Soto et al., 2018).

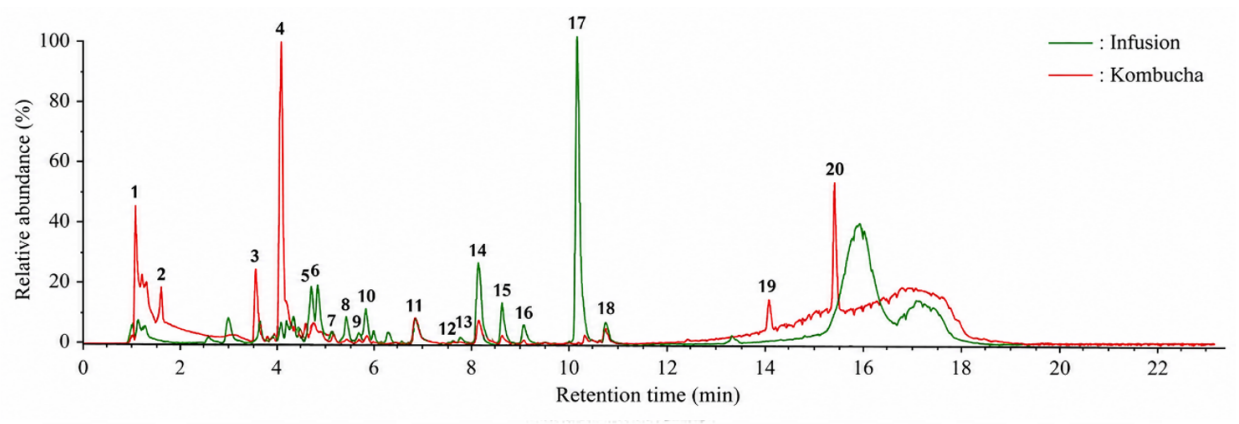


Fig. 1 LC-MS representative chromatograms for *S. caseolaris* leaf infusion and kombucha

Journal of Food Health and Bioenvironmental Science (September – December 2026), 19(3), e269550
https://doi.org/10.65763/jfhb.2026.e269550

Table 2 Tentative identification of compounds in *S. caseolaris* leaf infusion and its kombucha counterpart

No.	RT (min)	Observed m/z	Theoretical m/z	Mass error (ppm)	Molecular formula	Major fragment ions (m/z)	Tentative identification	Class	References
1	1.261	381.0822	381.0820	0.5	$C_{17}H16O_{10}$	365.105,360.150, 343.124, 219.030, 203.053	Gallic acid derivative	Phenolic compound	Wang et al. (2021); Yan et al. (2022)
2	1.717	423.0927	423.0920	1.7	$C_{19}H_{18}O_{11}$	402.161, 360.150, 343.124, 205.071	Mangiferin	Xanthone	Eff et al. (2023)
3	3.671	307.0818	307.0820	–0.7	$C_{15}H_{14}O_7$	289.070, 151.039, 139.039	Epigallocatechin	Flavan-3-ol	Mizooku et al. (2003)
4	4.178	595.1650	–	–	$C_{27}H_{30}O_{15}$	195.087, 138.066	Flavone diglycoside derivative	Flavone glycoside	–
5	4.797	611.1612	611.1607	0.8	$C_{27}H_{30}O_{16}$	595.169, 433.115, 313.072, 287.056	Rutin	Flavonol glycoside	Chen et al. (2015)
6	4.923	449.1100	449.1080	4.5	$C_{21}H_{20}O_{11}$	287.057, 433.116, 219.103	Luteolin-O-glucoside isomer	Flavone glycoside	Nguyen et al. (2024)
7	5.211	449.1100	449.1080	4.5	$C_{21}H_{20}O_{11}$	287.056, 433.114, 271.061	Luteolin-7-O-glucoside	Flavone glycoside	Nguyen et al. (2024); Sadhu et al. (2006)
8	5.500	449.1080	449.1080	0.0	$C_{21}H_{20}O_{11}$	287.055, 433.114, 303.055, 271.060, 219.102	Quercitrin	Flavonol glycoside	Audah et al. (2022)
9	5.739	271.0606	271.0601	1.8	$C_{15}H_{10}O_5$	223.133, 191.143, 133.101	Apigenin	Flavone	Dahibhate et al. (2018)
10	5.872	303.0500	303.0507	–2.3	$C_{15}H_{10}O_7$	197.117, 179.107, 219.100	Quercetin	Flavonol	Dahibhate et al. (2018)
11	6.906	287.0560	287.0550	3.5	$C_{15}H_{10}O_6$	195.102, 247.170	Luteolin	Flavone aglycone	Nguyen et al. (2024); Sadhu et al. (2006)
12	7.714	633.2930	–	–	–	601.267, 595.216, 441.226, 425.252, 271.060	Luteolin-O-rutinoside-like compound	Flavone glycoside	Nguyen et al. (2024)
13	7.848	462.2130	–	–	–	425.252, 351.215, 287.054, 195.088, 189.091	Flavonoid-related compound	Phenolic/Flavonoid-related compound	–
14	8.178	351.2150	351.2166	–4.6	$C_{20}H_{30}O_5$	275.201, 293.212, 195.138	Oxygenated diterpenoid derivative	Terpenoid	Cerri and Galli (2025)
15	8.664	353.2300	353.2323	–6.4	$C_{20}H_{32}O_5$	369.204, 295.227, 277.216, 195.138	Oxygenated diterpenoid-like compound	Terpenoid	Cerri and Galli (2025)
16	9.099	205.1230	205.1223	3.4	$C_{11}H_{16}O_4$	181.123, 163.112, 118.065	Phenolic acid derivative	Phenolic compound	Cerri and Galli (2025)
17	10.196	274.2750	–	–	–	256.264, 215.016, 153.138	Sterol-related compound	Steroid/Sterol derivative	–
18	10.773	214.2540	–	–	–	274.275, 333.204, 195.089	Sterol-related compound	Steroid/Sterol derivative	–
19	14.112	358.3690	–	–	–	529.463, 546.490, 574.521	Sterol-related compound	Steroid	–
20	15.447	282.2800	–	–	–	265.253, 304.261	Fatty acid amide-like compound	Lipid-related compound	–

4. Antioxidant activity (DPPH radical scavenging assay)

The antioxidant potential of *S. caseolaris* leaf infusion and its fermented counterpart was evaluated using the DPPH radical scavenging assay, and the results are expressed as IC_{50} values (Table 1). The unfermented infusion exhibited an IC_{50} value of 46.05±0.12 µg/mL, while the fermented sample showed a slightly lower IC_{50} value of 45.26±0.26 µg/mL. Although fermentation resulted in a numerical decrease in IC_{50}, statistical analysis indicated that the difference between the infusion and the fermented sample was not significant ($p>0.05$). In contrast, the reference antioxidant gallic acid exhibited significantly stronger activity with an IC_{50} value of 38.28±1.60 µg/mL ($p<0.05$).

When compared with previously reported extracts of *S. caseolaris* leaves, the antioxidant activity observed in this study falls within a moderate range. Previous studies have reported that ethanol extracts of *S. caseolaris* leaves exhibited very strong antioxidant activity, with an IC_{50} value of 4.2499±3.0506 ppm and radical scavenging activity reaching 80.21% at a concentration of 2.5 mg/mL (Audah et al., 2022; Pagarra et al., 2022). In contrast, water extracts showed substantially weaker antioxidant

activity with an IC_{50} of approximately 89 mg/mL (Kartikaningsih et al., 2025). These differences highlight the strong influence of extraction solvents on the recovery of phenolic antioxidants, with organic solvents generally extracting a broader range of bioactive compounds than aqueous preparations.

The absence of a statistically significant improvement suggests that the modest increase in total phenolic and flavonoid contents observed after fermentation did not translate into a measurable enhancement of DPPH radical scavenging capacity under the tested conditions. Antioxidant activity is influenced not only by the total concentration of phenolic compounds but also by the structural characteristics of individual molecules. In particular, the number and position of hydroxyl groups, the degree of conjugation within aromatic rings, and the presence of glycosidic substitutions can significantly affect radical scavenging efficiency and electron-donating ability (Heim et al., 2002; Rice-Evans et al., 1996). Consequently, alterations in the phenolic composition caused by fermentation do not necessarily correspond to proportional changes in antioxidant activity.

Previous studies on kombucha fermentation of plant substrates have reported variable effects on antioxidant capacity, depending on the composition of the starting material and the extent of microbial metabolism (Jayabalan et al., 2014; Laavanya et al., 2021). In the present study, fermentation may have altered the phytochemical matrix, as indicated by UPLC–MS profiling; however, these compositional changes did not result in a significant increase in radical scavenging activity. Overall, the results indicate that while SCOBY fermentation modified the phytochemical environment of the *S. caseolaris* leaf infusion, its contribution to DPPH-based antioxidant activity remained comparable to that of the unfermented preparation.

5. Antibacterial efficacy of *S. caseolaris* kombucha

The antibacterial activity of *S. caseolaris* leaf infusion and its fermented kombucha was evaluated against *S. aureus* ATCC 25923, *P. acnes* ATCC 6919, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 using the disk diffusion method. The results are summarized in Table 3. The unfermented *S. caseolaris* leaf infusion did not exhibit detectable antibacterial activity against any of the tested microorganisms at concentrations up to 5%. In contrast, the fermented preparation demonstrated inhibitory activity against *P. aeruginosa* and *S. aureus.* At a concentration of 2.5%, the kombucha preparation produced an inhibition zone of 10±2.00 mm against *P. aeruginosa,* which increased to 16±2.00 mm at 5%. Additionally, the fermented sample exhibited moderate inhibition against *S. aureus* at 5% concentration, with an inhibition zone of 7.33±1.15 mm.

Table 3 Antibacterial activity (inhibition zones, mm±SD) of *S. caseolaris* leaf infusion and kombucha

Sample	Concentration (%)	Inhibition zone (mm±SD)			
		Gram-negative		Gram-positive	
		E. coli	*P. aeruginosa*	*P. acnes*	*S. aureus*
S. caseolaris leaf infusion	1.25	–	–	–	–
	2.5	–	–	–	–
	5	–	–	–	–
S. caseolaris leaf kombucha	1.25	–	–	–	–
	2.5	–	10±2.00	–	–
	5	–	16±2.00	–	7.33±1.15
Chloramphenicol	0.3	31.86±0.03	30.66±0.57	18.42±0.25	56.46±0.43
DMSO	–	–	–	–	–

The phytochemical profile obtained from UPLC–MS analysis suggests the presence of several phenolic and flavonoid derivatives that have previously been associated with antimicrobial activity, including compounds structurally related to epigallocatechin, mangiferin, and flavone glycosides. These compounds were tentatively identified based on accurate mass measurements and characteristic fragmentation patterns. Although the present study did not quantify individual metabolites or directly evaluate their antibacterial mechanisms, previous studies have shown that such compounds may inhibit bacterial growth through membrane disruption, interference with enzyme systems involved in fatty acid biosynthesis, or modulation of bacterial metabolism pathways (Cushnie & Lamb, 2005; Zhang & Rock, 2004). The combined presence of flavonoids, phenolic derivatives, and fermentation-derived metabolites may collectively contribute to the antibacterial properties observed in the fermentation preparation.

In addition to phytochemical modification, acidification during fermentation may also contribute to the observed antibacterial activity. The pH of the infusion decreased from 4.0 to 3.0 following fermentation, indicating the production of organic acids typically generated during kombucha fermentation, such as acetic and gluconic acids. These acids are known to inhibit bacterial growth by disrupting proton gradients across bacterial membranes and altering intracellular pH homeostasis (Jayabalan et al., 2014; Sreeramulu et al., 2000). Consequently, the antibacterial activity of the fermented preparation may result from the combined influence of phytochemical changes and increased acidity.

Journal of Food Health and Bioenvironmental Science (September – December 2026), 19(3), e269550
https://doi.org/10.65763/jfhb.2026.e269550

The antibacterial activity observed in the fermented preparation can be compared with previous reports on *S. caseolaris* extracts. Earlier studies have shown that leaf extracts of *S. caseolaris* exhibit variable antibacterial potency depending on the solvent system used. For instance, several solvent fractions of leaf extracts produced inhibition zones ranging from approximately 7.5 to 8.5 mm against *S. aureus* and around 7.5 mm against *E. coli* in agar diffusion assays (Cerri & Galli, 2025). In another study using ethanol extracts, stronger antibacterial activity was reported, with inhibition zones exceeding 25 mm against certain bacterial species and minimum inhibitory concentration (MIC) values as low as 0.04–0.06 mg/mL (Nguyen et al., 2024). The stronger activity observed in organic solvent extracts may be attributed to the higher extraction efficiency of non-polar or moderately polar antimicrobial constituents.

Compared with these findings, the inhibition zones observed in the present study (up to 16 mm against *P. aeruginosa* and 7.33 mm against *S. aureus* at 5% concentration) indicate moderate antibacterial activity. The relatively lower activity may be attributed to the use of a water-based infusion, which extracts fewer non-polar antimicrobial compounds than organic solvent extracts. Nevertheless, the emergence of antibacterial activity only after fermentation suggests that SCOBY fermentation may enhance the bioactivity of the aqueous preparation by modifying phytochemical composition and producing additional antimicrobial metabolites. Overall, these results suggest that fermentation can partially improve the biological activity of *S. caseolaris* aqueous preparations, although the antibacterial potency remains lower than that reported for concentrated organic extracts.

Conclusion

This study demonstrates that SCOBY fermentation can improve the functional of *S. caseolaris* leaf infusion. Fermentation reduced pH and decreased bitterness while producing a mild sour taste, indicating improved palatability of the beverage. Although only minor changes in antioxidant activity were observed, fermentation resulted in the emergence of antibacterial activity, particularly against *P. aeruginosa* and *S. aureus,* whereas the unfermented infusion showed no detectable inhibition. UPLC–MS profiling suggested qualitative changes in the phytochemical composition of the infusion, indicating that microbial biotransformation during fermentation may alter the phenolic matrix and contribute to the observed bioactivity. These findings highlight the potential of kombucha fermentation as a mild bioprocessing strategy to enhance the functional value of mangrove-derived plant infusions. However, the antibacterial activity observed remains moderate compared with previously reported solvent extracts of *S. caseolaris*. Future studies involving metabolite quantification, structural confirmation of bioactive compounds, and more comprehensive antimicrobial evaluation, such as MIC determination, will be necessary to further clarify the pharmacological potential of fermented *S. caseolaris* products.

Acknowledgment

The authors gratefully acknowledge the financial support provided by the Faculty of Medicine and Health Sciences, Universitas Jambi, through the Novice Lecturer Research Grant (Contract No. 343/UN21.11/PT.01.05/SPK/2025).

Conflicts of interest

The authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT to assist with language editing and grammar improvement. The authors carefully reviewed and revised the generated text and take full responsibility for the accuracy, integrity, and originality of the final manuscript.

References

Abeysinghe, P.D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. *Indian Journal of Pharmaceutical Sciences, 72*(2), 167. https://doi.org/10.4103/0250-474X.65019

Antolak, H., Piechota, D., & Kucharska, A. (2021). Kombucha tea—a double power of bioactive compounds from tea and symbiotic culture of bacteria and yeasts (SCOBY). *Antioxidants, 10*(10), 1541. https://doi.org/10.3390/ANTIOX10101541

Arung, E.T., Kuspradini, H., Kusuma, I.W., Bang, T.H., Yamashita, S., Katakura, Y., & Shimizu, K. (2015). Effects of isolated compound from *Sonneratia caseolaris* leaf: A validation of traditional utilization by melanin biosynthesis and antioxidant assays. *Journal of Applied Pharmaceutical Science, 5*(10), 039–043. https://doi.org/10.7324/JAPS.2015.501007

Audah, K.A., Batubara, R., Julkipli, Wijaya, E., Kurniawaty, E., & Batubara, I. (2020). Antibacterial screening of mangrove extract library showed potential activity against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Tropical Life Science, 10*(2), 105–111. https://doi.org/10.11594/JTLS.10.02.03

Audah, K.A., Ettin, J., Darmadi, J., Azizah, N.N., Anisa, A.S., Hermawan, T.D.F., & Batubara, I. (2022). Indonesian mangrove *Sonneratia caseolaris* leaves ethanol extract is a potential super antioxidant and anti-methicillin-resistant *Staphylococcus aureus* drug. *Molecules, 27*(23), 8369. https://doi.org/10.3390/molecules 27238369

Bandaranayake, W.M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management, 10*(6), 421–452. https://doi.org/10.1023/A:1021397624349

Barakat, N., Beaufort, S., Rizk, Z., Bouajila, J., Taillandier, P., & El Rayess, Y. (2022). Kombucha analogues around the world: A review. *Critical Reviews in Food Science and Nutrition, 63*(29), 10105–10129. https://doi.org/10.1080/10408398.2022.2069673

Cerri, F., & Galli, P. (2025). Phytochemistry and pharmacological potential of the mangrove plant *Sonneratia caseolaris:* A comprehensive review. *Marine Drugs, 23*(10), 378. https://doi.org/10.3390/MD23100378

Chen, C., & Liu, B.Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology, 89*(5), 834–839. https://doi.org/10.1093/chromsci/bmu078

Chen, M., Zhang, X., Wang, H., Lin, B., Wang, S., & Hu, G. (2015). Determination of rutin in rat plasma by ultra performance liquid chromatography tandem mass spectrometry and application to pharmacokinetic study. *Journal of Chromatographic Science, 53*(4), 519–525. https://doi.org/10.1093/chromsci/bmu078

Cushnie, T.T., & Lamb, A.J. (2005). Antimicrobial activity of flavonoids. International *Journal of Antimicrobial Agents, 26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dahibhate, N.L., Roy, U., & Kumar, K. (2018). Phytochemical screening, antimicrobial and antioxidant activities of selected mangrove species. *Current Bioactive Compounds, 16*(2), 152–163. https://doi.org/10.2174/1573407214666180808121118

Diez-Ozaeta, I., & Astiazaran, O.J. (2022). Recent advances in kombucha tea: Microbial consortium, chemical parameters, health implications and biocellulose production. *International Journal of Food Microbiology, 377,* 109783. https://doi.org/10.1016/j.ijfoodmicro.2022.109783

Efendi, M.R., Bakhtiar, A., Rusdi, M.S., & Putra, D.P. (2024). Comparative study of antibacterial activity of *Elephantopus scaber* Linn. and *Elephantopus mollis Kunth.* extract. *Current Applied Science and Technology, 24*(2), e0258350–e0258350. https://doi.org/10.55003/cast.2023.258350

Eff, A.R.Y., Huri, H.Z., Radji, M., Mun'im, A., Suyatna, F.D., & Eden, Y. (2023). Angiotensin converting enzyme (ACE) inhibitors activity from purified compounds fructus *Phaleria macrocarpa* (Scheff) Boerl. *BMC Complementary Medicine and Therapies, 23*(1), 56. https://doi.org/10.1186/s12906-023-03889-X

Halifah, P., Hartati, Rachmawaty, Yusminah, H., & Roshanida, A.R. (2019). Phytochemical Screening and antimicrobial activity from *Sonneratia caseolaris* fruit extract. *Materials Science Forum, 967,* 28–33. https://doi.org/10.4028/www.scientific.net/msf.967.28

Harun, A., Ab Rahim, N.E.A., Abd Jalil, M.A., Rosdi, A.M., Daud, S., Harith, S.S., Soâ, S.Z.M. & Hassan, N.M. (2016). Comparative study of antioxidant and antimicrobial activity of root, stem and leaves of *Leea indica species. Malaysian Journal of Science, 35*(2), 259–274. https://doi.org/10.22452/mjs.vol35no2.12

Heim, K.E., Tagliaferro, A.R., & Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry, 13*(10), 572–584. https://doi.org/10.1016/S0955-2863(02)00208-5

Ismed, F., Desti, W.N., Arifa, N., Rustini, R., & Putra, D.P. (2021). TLC-Bioautographic and LC-MS/MS detection of antimicrobial compounds from four semipolar extracts of *Cladonia Species.* In *Proceedings of 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021* (ICCSCP 2021) (pp. 49-59). Atlantis Press. https://doi.org/10.2991/ahsr.k.211105.008

Jayabalan, R., Malbaša, R.V., Lončar, E.S., Vitas, J.S., & Sathishkumar, M. (2014). A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 538–550. https://doi.org/10.1111/1541-4337.12073

Joevanda, S., Salsabilla, N., Qiftiah, M., Sani, F., Rusdi, M.S., & Efendi, M.R. (2024). Formulation of an antibacterial peel-off gel mask from kombucha fermented with Gerga orange peel infusion (*Citrus x aurantium* L.). I*ndonesian Journal of Pharmaceutical Education, 4*(3), 461–472. https://doi.org/10.37311/ijpe.v4I3.28769

Kalor, J.D., Wanimbo, E., & Indrayani, E. (2025). A systematic review of bioactive compounds, traditional uses, and conservation perspectives of *Sonneratia caseolaris. International Journal of Pharmaceutical and Bio-Medical Science, 5*(5), 350–357. https://doi.org/10.47191/ijpbms/v5-i5-10

Kartikaningsih, H., Fitriana, N., Anggraeni, I.L., Semedi, B., & Pertiwi Koentjoro, M. (2025). The potential of *Sonneratia caseolaris* mangrove leaves extract as a bioactive food ingredient using various water extract. *F1000Research, 13,* 249. https://doi.org/10.12688/

f1000research.143708.4

Kathiresan, K., & Bingham, B.L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology, 40,* 81–251. https://doi.org/10.1016/s0065-2881(01)40003-4

Kundu, P., Debnath, S.L., Ahad, M.F., Devnath, H.S., Saha, L., Karmakar, U.K., & Sadhu, S.K. (2023). Exploration of *in vivo* and *in vitro* biological effects of *Sonneratia caseolaris* (L.) fruits supported by molecular docking and ADMET study. *BioMed Research International, 2023*(1), 4522446. https://doi.org/10.1155/2023/4522446

Laavanya, D., Shirkole, S., & Balasubramanian, P. (2021). Current challenges, applications and future perspectives of SCOBY cellulose of kombucha fermentation. *Journal of Cleaner Production, 295,* 126454. https://doi.org/10.1016/j.jclepro.2021.126454

Leal, J.M., Suárez, L.V., Jayabalan, R., Oros, J.H., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. *CyTA - Journal of Food, 16*(1), 390–399. https://doi.org/10.1080/19476337.2017.1410499

Liu, B., Wang, X., Wang, Y., Chen, X., Jin, X., & Luo, X. (2023). Review of compounds and activities from mangrove Sonneratia genus and their endophytes. *Journal of Holistic Integrative Pharmacy, 4*(3), 218–227. https://doi.org/10.1016/j.jhip.2023.11.003

Ministry of Health Indonesia. (2017). *Farmakope Herbal Indonesia* (2nd ed.). Directorate General of Pharmaceutical and Medical Devices, Ministry of Health. https://farmalkes.kemkes.go.id/unduh/farmakope-herbal-indonesia-edisi-ii-tahun-2017/

Mizooku, Y., Yoshikawa, M., Tsuneyoshi, T., & Arakawa, R. (2003). Analysis of oxidized epigallocatechin gallate by liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry, 17*(16), 1915–1918. https://doi.org/10.1002/rcm.1135

Nguyen, L.T.T., Nguyen, T.T., Nguyen, H.N., & Bui, Q.T.P. (2024). Analysis of active compounds and bioactivity of leaves extracts of Sonneratia species. *Engineering Reports, 6*(10), e12870. https://doi.org/10.1002/eng2.12870

Pagarra, H., Rahman, R.A., Hartati, Rachmawaty, Hala, Y., & Esivan, S.M.M. (2022). Phytochemical screening, antimicrobial and antioxidant activity from *Sonneratia caseolaris* leaves extract. *Journal Teknologi, 84*(5), 59–66. https://doi.org/10.11113/jurnalteknologi.v84.17647

Rao, G.M.N. (2020). Flora of mangrove species utilized for ethnomedicinal practices in Gautami Godavari Estuary, Andhra Pradesh, India. In Khasim, S.M., Long, C., Thammasiri, K., & Lutken, H. (Eds.), *Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation* (pp. 97–107). Springer. https://doi.org/10.1007/978-981-15-1636-8_5

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933–956. https://doi.org/10.1016/0891-5849(95)02227-9

Rusdi, M.S., Febrianti, T.L., Al-Annur, Y., Sita, S.K., J., S.A., Ardita, M., Saputri, C.E., Efendi, M.R., Hisran, Halimatussa' diyah, Supriadi, Agusriani & Sugiarti. (2025). Antioxidant and antihyperglycemic effects of bay leaf (*Syzygium polyanthum* [Wight.] Walp.) kombucha in alloxan-induced diabetic mice. *Science Essence Journal, 41*(2), 168–179. https://ejournals.swu.ac.th/index.php/sej/article/view/17002

Sadhu, S.K., Ahmed, F., Ohtsuki, T., & Ishibashi, M. (2006). Flavonoids from Sonneratia caseolaris. *Journal of Natural Medicines, 60*(3), 264–265. https://doi.org/10.1007/s11418-006-0029-3

Simlai, A., Rai, A., Mishra, S., Mukherjee, K., & Roy, A. (2014). Antimicrobial and antioxidative activities in the bark extracts of *Sonneratia caseolaris,* a mangrove plant. *EXCLI Journal, 13,* 997-1010. https://pmc.ncbi.nlm.nih.gov/articles/pmc4464296/

Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *Journal of Agricultural and Food Chemistry, 48*(6), 2589–2594. https://doi.org/10.1021/jf991333m

Tran, T. khanh, Ha, P.T.T., Henry, R.J., Nguyen, D.N.T., Tuyen, P.T., & Liem, N.T. (2024). Polyphenol contents, gas chromatography-mass spectrometry (GC–MS) and antibacterial activity of methanol extract and fractions of *Sonneratia caseolaris* fruits from Ben Tre province in Vietnam. *Journal of Microbiology and Biotechnology, 34*(1), 94–102. https://doi.org/10.4014/jmb.2304.04019

Villarreal-Soto, S.A., Beaufort, S., Bouajila, J., Souchard, J.P., & Taillandier, P. (2018). Understanding kombucha tea fermentation: A review. *Journal of Food Science, 83*(3), 580–588. https://doi.org/10.1111/1750-3841.14068

Wang, Z., Wu, Z., Zuo, G., Lim, S.S., & Yan, H. (2021). Defatted seeds of Oenothera biennis as a potential functional food ingredient for diabetes. *Foods, 10*(3), 538. https://doi.org/10.3390/foods10030538

Yan, G., Zheng, P., Weng, S., Zhang, Y., Xu, W., Luo, J., Fei, J., Wang, J., Zhang, H., Hu, H. & Sun, B. (2022). Comparison of chemical compositions and antioxidant activities of fresh and dried *Rosa roxburghii* Tratt fruit. *Natural Product Communications, 17*(4), 1934578X221095350. https://doi.org/10.1177/1934578x221095350

Zhang, Y.M., & Rock, C.O. (2004). Evaluation of epigallocatechin gallate and related plant polyphenols as inhibitors of the FabG and FabI reductases of bacterial type II fatty-acid synthase. *Journal of Biological Chemistry, 279*(30), 30994–31001. https://doi.org/10.1074/jbc.m403697200