Kombucha – An ancient fermented beverage with desired bioactivities: A narrowed review

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Kombucha, originated in China 2000 years ago, is a sour and sweet-tasted drink, prepared traditionally through fermentation of black tea. During the fermentation of kombucha, consisting of mainly acidic compounds, microorganisms, and a tiny amount of alcohol, a biofilm called SCOBY forms. The bacteria in kombucha has been generally identified as Acetobacteraceae. Kombucha is a noteworthy source of B complex vitamins, polyphenols, and organic acids (mainly acetic acid). Nowadays, kombucha is tended to be prepared with some other plant species, which, therefore, lead to variations in its composition. Pre-clinical studies conducted on kombucha revealed that it has desired bioactivities such as antimicrobial, antioxidant, hepatoprotective, anti-inflammatory, etc. Only a few clinical studies have been also reported. In the current review, we aimed to overhaul pre-clinical bioactivities reported on kombucha as well as its brief compositional chemistry. The literature data indicate that kombucha has valuable biological effects on human health.

1. Introduction

Kombucha, as one of the popular drinks of recent years, is a fermented product that first became widespread in China, traditionally using black or green tea [Camellia sinensis (L.) Kuntze] as substrates. When it started to become popular in Japan, it was named “kombucha” created with the combination of the word “kombu” and “cha”, meaning alga and tea, respectively. Kombucha is commercially sold as a tea-type beverage in markets, while it can be also prepared at home. In traditional way of preparation, black or green tea is fermented with 5–10% sucrose (Emilianowicz & Malinowska-Panczyk, 2020; Massoud et al., 2022). According to its standard practice, black or green tea is left to infuse with sucrose for 5 min and then cooled to room temperature. After the cooled product is filtered, kombucha liquid obtained from the previous production is added. This liquid is a biofilm called “mother” or “SCOBY” (Symbiotic Community of Bacteria and Yeast), which is a...
mixture of bacteria, especially from Acetobacteraceae family (Acetobacter acetii, A. estuensis, A. pasteuriensis, Gluconobacter oxydans, Komagataeibacter kombuchae, K. rheaticus, and K. xylinus), Lactobacillus sp. as well as osmophilic yeasts (Brettanomyces/Dekkera, Candida, Saccharomyces, Schizosaccharomyces, Starmerella sp., Torulopsis, Pichia sp., and Zygosaccharomyces) (Kaashyap et al., 2021; Tapias et al., 2022). The mixture is covered, then the fermentation process is completed by keeping it in the dark area for 10–14 days, and the product is also called “tea fungus”. Sucrose is used as a carbon source by these microorganisms. It is first hydrolyzed into glucose and fructose, where glucose is converted into ethanol and carbon dioxide by the microorganisms in the mixture. The ethanol formed turns into acetic acid with Acetobacter and the pH of the mixture decreases with acidity. In this procedure, tea extract functions as a nitrogen source, while organic acids and carbon dioxide are formed in the presence of oxygen with SCOBY, while microorganisms form a thick biofilm of cellulose (Fig. 1). The biofilm (SCOBY) occurs based on compliant and competitive interfaces among the microorganisms.

During COVID-19 pandemic, the consumption and demand of fermented beverages has increased in many countries as they have been reported to display antioxidant and other beneficial effects (La Torre et al., 2021). Therefore, our intention was to focus on desired biological activities of kombucha in connection to its brief compositional chemistry to present the current review through searching the databases including Web of Science (WoS), PubMed, and Scopus between 2016 and 2021.

2. Chemical composition of kombucha

After the fermentation process is completed, the main products formed in kombucha are ethanol, gluconic acid, and acetic acid. In addition to these compounds, it contains tea polyphenols (catechins, theaflavins, and flavanols) since the substrate is tea, organic acids (e.g. folic, lactic, carbonic, glucuronic, oxalic, malic, malonic, tartaric, succinic, pyruvic, citric, and usnic acids), water-soluble vitamins (e.g. B1, B2, B3, B6, B12, and C) as well as vitamin E, enzymes (amylase and invertase), amino acids, proteins, purines, and minerals (Kaashyap et al., 2021). The content of kombucha varies according to the fermentation time, the type, and the amount of bacteria and yeasts that play a critical role in fermentation. In a recent study, the ratio of gluconic acid was observed to change by using the synthetic microbial community (SMC) and the proportion of gluconic acid contained in kombucha was related to the taste quality (Li et al., 2022). It has been shown that the rate of phenolic compounds in kombucha prepared using black and green tea, which left to fermentation with tea residues without filtering, was 5.68 times higher than those designed by filtration (Zhou et al., 2022). The analyses performed by HPLC/ESI–MS in kombucha prepared with yerba mate (Ilex paraguariensis A.St.-Hil) showed presence of phenolic acids such as caffeoylquinic acid and dicaffeoylquinic acid, flavonoids, and xanthines. While the total phenolic compound ratio was highest on the 21st day of fermentation, it decreased significantly on the 35th day of fermentation (Ziemlewska et al., 2021).

Today, kombucha can be prepared using different herbal raw materials as the substrate. In a study, black tea, green tea, yerba mate, lavender, oregano, and fenell were used as substrates and the antioxidant activities of biofilms obtained by fermenting with the kombucha symbiotic mixture in presence of sucrose (100 g/L) were compared (Tapias et al., 2022). In the kombucha samples aforementioned, their pH values varied between 2.37 and 2.62, whereas the most acidic kombucha was found to belong to black tea-derived one. The same result was obtained with black tea-derived kombucha in terms of biofilm amount produced (10.3 ± 0.5 g/L), whereas the least amount of biofilm occurred with green tea (3.3 ± 0.2 g/L). The highest and lowest yields were obtained correspondingly with black tea-derived kombucha (0.39 ± 0.04 g/L) and yerba mate-derived kombucha (0.21 ± 0.01 g/L). However, the best antioxidant capacity as ascorbic acid equivalent was determined in yerba mate-derived kombucha (312.4 ± 14.2 mg/mL), black tea (48.3 ± 2.3 mg/mL) and lavender-derived (37.7 ± 1.6 mg/mL) kombuchas possessed the lowest antioxidant capacity.

Different fermentation temperatures and sugar ratios were used in a study by Vukmanovic et al. (2021), in which the effluent from the grape was used as a substrate. At the end of 9-day fermentation performed at 20, 25, and 30 °C, 70 g/L sugar was applied at 30 °C temperature. In the kombucha obtained, acetic acid was determined as the major acid, while oxalic acid was observed to occur at the highest rate at 20 °C.

*Porphyra dentata* Kjellman (laver), a red alga containing phycoerythrin, vitamin B12, phenolics, and flavonoid-derivative compounds, was used as the substrate to prepare kombucha (Aung & Eun, 2021). For preparation; two separate procedures were applied to extract the dry sheets of *P. dentata*. The first procedure involved pouring 100 °C water to the dry material and then infusing it for 15 min. The second procedure involved pouring water on the dry material and ultrasound-assisted extracting it at 80 °C for 20 min. Both extracts were used to make kombucha, which were then compared to each other. Kombucha was fermented at 25 °C for 14 days. During the fermentation, the samples obtained from both kombuchas at 0, 3rd, 7th, 10th, and 14th days were evaluated. The findings indicated that high temperature led to increase in the fermentation rate and acid accumulation, whereas a longer fermentation time caused a high acidity as well as a reduction in the number of bioactive components such as flavonoids and phenolic compounds. The amount of organic acids such as α-ketoglutaric and acetic acids was higher in kombucha prepared from the ultrasound-assisted laver extract.

Biological activities and total phenolic content were compared in kombucha using different percentages of *Curcuma longa* L. rhizome by Zubadiyah et al. (2021). According to multi-criteria decision-making (MCDM), the best result was observed in kombucha prepared at 0.8% concentration. It was found that kombuchas prepared by adding *Euphorbia uniflora* L. (pitanga) and *Spondias tuberosa* Arruda (umbu-caja) fruits had a sweeter flavor due to their high sugar content (da Silva et al., 2021). While acetic, butyric, citric, malic, and succinic acids were detected umbu-caja-flavored kombucha, whereas pitanga-derived kombucha contained all organic acids mentioned except for malic acid. Apart from these compounds, epigallocatechin gallate (EGCG), catechin, caftaric acid, and hesperidin were the main phenolic compounds, while...
curzerene and β-caryophyllene were the terpene derivatives detected. Actually, EGCG was the major compound found in both kombuchas over 63%.

The kombucha was prepared from the anthocyanin-rich plantCamellia sinensis var. assamica cv. Zijuan (Zijuan tea) in China turned pinkish due to the fact that anthocyanins turn red in an acidic environment (Zou et al., 2021). Anthocyanins determined using LC-MS analysis in Zijuan-derived kombucha were delphinidin-3-O-beta-D-galactoside, cyanidin-3-O-beta-D-galactoside, delphinidin-3-O-beta-D-[6-(E)-p-coumaroyl] galactopyranoside, and cyanidin-3-O-beta-D-[6-(E)-p-coumaroyl] galactopyranoside. In HPLC analysis, gluconic acid was the major organic acid, while succinic, citric, and gallic acids were identified as the minor ones. Volatile compounds were found at the highest rate in kombucha prepared from Zijuan tea. In another study comparing the fluoride contents of kombuchas prepared with 7 and 14 days of fermentation from white, black, green, and red tea, it was recommended to consume red and white tea-derived kombuchas made with 7-day fermentation with lower fluoride content to reduce exposure (Jakubczyk et al., 2021). The amount of acetic and lactic acids along with soluble proteins was found to escalate in kombucha prepared by adding Stevia rebaudiana (Bertoni) Bertoni leaves to green tea extract (Fereydouni et al., 2021). When vitamin C analysis was performed at different fermentation times (e.g., 7 and 14 days) in kombucha prepared from Annona muricata L. (soursop) leaves, vitamin C amount increased significantly during fermentation (Candra et al., 2021). Volatile compounds with different chemical structures (phenyl ethyl alcohol, octanoic, and decanoic acid ethyl esters, etc.) were detected in kombucha prepared with the aromatic plants, e.g. Chrysanthemum morifolium Ramat., Lonicera japonica Thunb., and Mentha spicata L. It has been concluded that aromatic plants can be used to obtain preferable kombuchas with pleasing flavors (Zhang et al., 2021). The content of kombuchas prepared from the medicinal mushroomsCoriolus versicolor (L.) Quel. [syn. Trametes versicolor (L.) Lloyd.] andLentinus edodes (Berk.) Pegler was compared. It was shown thatC. versicolor-type of kombucha had higher polysaccharide, phenolic, and flavonoid contents compared to the one prepared fromL. edodes (Sknepek et al., 2021). The chemical compositions of the green seed extract from Coffea arabica L. and kombucha prepared from the coffee beans were examined by HPLC-UV-ESI-MS and the decreased amounts of polyphenol, caffeine, and trigonelline were determined in kombucha (Zofia et al., 2020). Kombuchas were prepared fromC. sinensis (black and green tea), Mentha × piperita L., Urtica dioica L., Cyclobia oblonga Mill. leaves, Satureja montana L.,Thymus serpyllum L., andSambucus nigra L. at different fermentation periods (3–10 days) (Vitas et al., 2020). When the compounds of the final products were compared, the highest acetic acid amount was found inS. nigra-derived kombucha, while the highest total phenol and flavonoid contents were found inM. piperita-derived kombucha.

3. Preclinical outcomes on biological activities of kombucha

3.1. Antioxidant activity

Free radicals are known to be highly reactive due to the unpaired electron located in their structure and disrupt normal functions in the human body by taking electrons from structures such as DNA, RNA, lipids, and proteins. Acute and chronic diseases such as neurodegenerative diseases, cardiovascular diseases, cancer, liver diseases, skin aging, diabetes, and cataracts are known to be linked to oxidative stress induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Neha et al., 2019). A number of studies have been conducted to determine the antioxidant activity of various kombuchas and the compound(s) responsible for antioxidant activity. As aforementioned, black tea is the most commonly used substrate for preparing traditional kombucha. However, in order to obtain kombucha with different flavors or to increase antioxidant activity, different substrates such as assorted fruit juices, milk, wine or herbal teas (except black or green tea), can be used for fermentation. For instance, in a study conducted by Sun et al. (2015), kombucha was obtained by fermenting black tea and wheatgrass juice (WGJ) obtained by squeezing the ripe sprouts of wheat seeds (Triticum aestivum L.) at different rates. The SCOBY used for fermentation containedGlucosacetobacter rhaeticus andG. roseus as bacterial components in addition toDekkera bruxellensis as yeast component. In vitro tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzotiazoline-6-sulfonic acid) (ABTS) radical scavenging activity along with oxygen radical absorption capacity (ORAC) were performed to define in vitro antioxidant capacity of kombucha. The highest level of antioxidant capacity was observed in kombucha obtained WGJ:black tea (1:1) mixture for 3 h fermentation time. The amount of total phenols and flavonoids increased in proportion to the amount of WGJ, whereas the amount of total anthocyanin amount remained constant. As the percentage of WGJ increased, the content of gallic and caffeic acids as well as catechin decreased, while the content of ferulic and chlorogenic acids along with rutin augmented. According to the result of HPLC analysis, the most abundant polyphenols in traditional kombucha were determined as gallic and caffeic acids along with catechin (Sun et al., 2015). In another study (Gambas-Gomez et al., 2016), infusions of Euca lyptus camaldulensis Dehn. andLitchi glaucescens Kunth were used as substrates instead of black tea for preparation of kombucha. In kombucha fromL. glaucescens, the content of total flavonoids, flavonols, and phenols decreased significantly after fermentation, whereas the same compounds increased in kombucha fromE. camaldulensis. When the effect of the fermentation process on antioxidant activity was examined, it was found that DPPH radical scavenging activity, the degree of inhibition of lipid peroxidation in healthy human serum, and the clearance of nitric oxide (NO), which tends to form hydroxyl radicals, increased with the fermentation of both plant infusions. In contrast to other studies, this study, in which antioxidant activity increased despite a decrease in the total amount of phenols and flavonoids, concluded that polyphenols are not the only group of chemical substances that have antioxidant activity during fermentation in kombucha.

In a study conducted to determine the shelf life of home-made kombucha, it was shown that the storage time of kombucha prepared after one month of fermentation of sweetened black tea in a dark and cool (+4 °C) environment can be a maximum of 4 months (La Torre et al., 2021). The HPLC analysis disclosed that the polyphenols in the kombucha, organic acids except chlorogenic acid, caffeine, and EGCG decreased significantly after the 5th month. The pH value increased with a longer storage time. The reason behind was linked to absence of sugar in the environment as bacteria and yeasts use organic acids as a carbon source in kombucha. As the quantity of polyphenols and organic acids in the kombucha lessened from the 4th month, the antioxidant activity reduced considerably. In vitro determination of antioxidant capacity by DPPH radical anion and ABTS radical cation method exhibited that fermented black tea had about 70% higher antioxidant activity than unfermented sweetened black tea and this activity decreased after the 5th month. On the other hand, rooibos [Aspalathus linearis (Burm.f.) R. Dahlgren], black tea, and green tea leaves were used as substrates in Gaggìa et al.’s (2019) study. The antioxidant capacity of kombucha prepared with the same SCOBY after 14-day fermentation of the substrates was determined by DPPH radical scavenging activity and ferric-reducing antioxidant power (FRAP) methods. In both methods, for all 3 kombuchas, the highest antioxidant activity was found in the kombucha obtained after 7 days of fermentation. After 7 days of fermentation, the highest antioxidant activity (1.31 ± 0.07 mmol TE/g by DPPH assay; 1.75 ± 0.06 mmol Fe^3+ by FRAP assay) was observed, when green tea was used as substrate. On the other hand, the lowest antioxidant activity was observed in kombucha from the rooibos leaves (0.45 ± 0.03 mmol TE/g by DPPH assay; 0.52 ± 0.01 mmol Fe^3+ /g by FRAP assay). The amount of catechins (i.e. catechin, epicatechin, epigallocatechin, gallo catechin, epicatechin gallate, and EGCG) in 3 samples was determined by HPLC-MS/MS analysis and amount of catechins was found to
diminish with increasing fermentation duration. The total content of catechin in kombucha prepared from black tea after 7 and 14 days of fermentation was 0.99 and 0.464 mg/g, respectively. Since no catechin can be detected in the tea prepared from rooibos leaves, it can be assumed that it has lower antioxidant activity compared to other kombuchas.

Antioxidant activity of unfermented green coffee extract and kombucha prepared from green coffee fermented for 7, 14, and 28 days was searched using the DPPH radical scavenging method and the lowest activity was found in kombucha obtained after 7-day fermentation. Actually, the antioxidant activity increased with longer duration, but it was still not as high as that of the first green coffee extract. The DPPH radical scavenging activity of kombuchas with a fermentation period of 7, 14, and 28 days was determined to be about 20%, 30%, and 40%, respectively. Divergently, the antioxidant activity of the unfermented green coffee extract was about 50% using the same method. The result of this study also indicated that the lowest total phenol and flavonoid contents were observed in 7-day-fermented kombucha, while increased with extended fermentation time, which parallels the antioxidant activity. The reason was thought to be the polymerization of phenols and flavonoids under the influence of fermentation. Increase in total amount of phenols and flavonoids was explained by depolymerization of the polymerized substances with extended fermentation time. This hypothesis was also supported by organoleptic analysis. For instance, turbidity was observed in kombucha fermented for 7 days, the turbidity decreased with increasing fermentation time. The reason was suggested that as polymerized and large structures formed a cloudy appearance as water solubility decreases (Zofia et al., 2020). In a study by Ivanisova et al. (2020), antioxidant capacity was determined by trolox equivalent antioxidative activity. The authors concluded that higher antioxidant activity after fermentation was related to the distinct rise in total phenolic amounts. In this regard, when total phenolic amounts were assumed to be due to substances released during fermentation, such as polyphenols, as a result of the analysis, it has been reported that the substances that contribute to the antioxidant activity of kombucha can be sorted as water-soluble vitamins (i.e., B1, B2, B6, B12, C), organic acids (i.e., acetic, gluconic, gluconuronic, lactic, tartaric, citric, and malic acids). It has also been shown to contain formic acid, etc. as well as minerals such as manganese, iron, nickel, copper, zinc, lead, cobalt, chromium, cadmium, etc. (Ivanisova et al., 2020; La Torre et al., 2021).

### 3.2. Anti-aging activity

Skin aging is a biological process characterized by skin dryness and wrinkles that develop with genetic and cellular aging as an intrinsic factor along with extrinsic factors, i.e., UV exposure, diet, smoking, stress, using a wrong skin product, etc. (Mesa-Arango et al., 2017). The flavonoids in black tea used in traditional kombucha preparation have anti-aging activity. In vitro and in vivo studies have shown that flavones may increase collagen synthesis, support DNA repair, protect against UVB radiation, and increase skin elasticity. Similarly, flavonoids have been shown to decrease secretion of senescence-associated secretory phenotype (SASP) and degradation of collagen and hyaluronic acid (Domaszewska-Szostek et al., 2021). In one study, fractions of black tea were administered intradermally to female NMRI mice at doses of 5 and 10 mg/mL, separately, for 14 days. After the mice were sacrificed, sections of the dermis and epidermis were collected. As a result of the examinations, it was found that the amount of type-I collagen and NAD⁺/NADH increased by 29.3% and about 50%, respectively, in the mice compared with the control group. Vitamin B3 in kombucha is the precursor of nicotinamide adenine dinucleotide (NAD). It is hypothesized that NAD decreases during cell aging, resulting in decreased collagen expression. In addition, vitamin B3 stimulates epidermal ceramide synthesis and helps to eliminate hyperpigmentation. Thus, it is an agent used in the treatment of skin blemishes (Pakravan et al., 2018).

In a study investigating the inhibition of the enzymes, e.g., elastase and collagenase, by kombucha obtained by fermentation of green coffee with a kombucha consortium, it was found that inhibition of collagenase decreased with the fermentation effect, while inhibition of elastase boosted (Zofia et al., 2020). The highest collagenase inhibition was found in the unfermented green coffee extract at a concentration of 1000 µg/mL with about 45% inhibition. Extract of kombucha obtained by 14-day fermentation inhibited collagenase at a dose of 1000 µg/mL at about 30%. The extracts of kombucha fermented for 7, 14, and 28 days displayed approximately similar inhibition against elastase at 100 µg/mL. In fact, there was no significant correlation between fermentation duration and the increase in these effects. In the same study, the effect of fermentation time on sun protection factor (SPF) was also investigated. SPF improved with a longer fermentation time. In fact, all these effects are assumed to be due to substances released during fermentation, polymerization or depolymerization. This is because the total amount of phenol and flavonoids first decreased significantly with fermentation and then increased again. This indicates polymerization and depolymerization of the molecules during fermentation. Although it is not known exactly from which component the anti-aging effect of kombucha originates and what the mechanism of action is, some estimates can be made. Flavonoids present in black tea have already been established to have an anti-aging effect. After fermentation with SCOBY, substances with anti-aging activities such as lactic acid as well as vitamins B3 and C are produced. Lactic acid is a member of the group of alpha-hydroxy acids (AHA) with the smallest molecular weight after glycolic acid. It exerts anti-aging activity by stimulating the biosynthesis of collagen and hyaluronic acid. Based on the hypothesis that NAD⁺ decreases during cell aging and leads to a decrease in collagen expression, vitamin B3 in kombucha may stimulate the biosynthesis of collagen and hyaluronic acid.
collagen biosynthesis in addition to vitamin C, which was revealed to possess antioxidant effect towards free radicals caused by UVB rays as well as stimulation of collagen biosynthesis. Thus, it was found to display an anti-aging effect by preventing the formation of wrinkles (Rattanawiwatpong et al., 2020).

3.3. Antimicrobial activity

A number of studies have been conducted on antimicrobial effect of kombucha (Tables 1 and 2), for some to find out the component(s) responsible for the mentioned activity, while several of them have investigated a possible link between acetic acid, a major component of kombucha, and antimicrobial activity. In one of these studies, the antibacterial activity of kombucha prepared from green and black teas was determined by agar diffusion test against Gram-positive and Gram-negative strains including Staphylococcus epidermidis (CIP 106510), S. aureus (ATCC 25923), Micrococcus luteus (NCIMB 8166), Escherichia coli (ATCC 35218), Pseudomonas aeruginosa (ATCC 27853), S. typhimurium (LT2), and Listeria monocytogenes (ATCC 19115). In order to determine the compound(s) responsible for antibacterial activity; unfermented tea, kombucha fermented at room temperature for 21 days, kombucha neutralized with 1 M NaOH (pH: 7.0) and tea with the same pH adjusted with acetic acid as kombucha were used. Tea denaturing at 120 °C for 20 min was prepared. Unfermented black tea and green tea had no effect against most bacterial strains. In contrast, the antimicrobial effect was seen in fermented teas, when these teas are neutralized as follows, black tea except S. aureus and M. luteus; the effect of green tea on all strains was lost except M. luteus and S. epidermidis. In general, as kombucha was neutralized, the antibacterial effect was significantly reduced. This consequence points out to the fact that the acidic character of fermented tea contributes to the antimicrobial effect. By denaturing the fermented tea in the same way, antimicrobial effect of kombucha prepared with black tea was lost only against L. monocytogenes. The effect of kombucha prepared from green tea and black tea continued on all other strains. However, it was observed that the antimicrobial activity decreased compared to fermented tea. This result indicated that antimicrobial effect is not only due to acetic acid or other organic acids (lactic acid, gluconic acid, etc.), but also to other biologically active components such as proteins and enzymes (Yuniarto et al., 2016). In the same study, the antifungal effect of kombucha on fungal species, i.e. Candida albicans, C. krusei, C. tropicalis, C. parapsilosis, C. glabrata, C. dubliniensis, and C. sake isolated from patients, was investigated. Antifungal activity was observed to rise with fermentation. While unfermented black tea showed no inhibition on the fungal lineage of C. dubliniensis, the zone of inhibition of kombucha prepared by fermentation of black tea was determined to be 12.0 ± 1.4 mm on the same pathogenic fungus by agar diffusion assay. Strong antifungal activity was observed against most fungal lineages with the kombucha prepared by fermentation of black tea. Unfermented green and black tea had no effect on fungal lineages except C. glabrata and C. sake. When kombucha was neutralized by 1 M NaOH or denatured by heat, activity on most fungi was reduced or lost altogether. In a study conducted on different strains, it has been displayed that the antifungal effect of kombucha prepared from black tea on Aspergillus flavus, C. albicans, and Microsporum gypseum was shown to be inversely proportional to the fermentation time of the kombucha. The inhibition zones on A. flavus by kombucha were measured 16.83 ± 0.05, 15.43 ± 0.25, and 11.06 ± 0.35 mm after 6, 12, and 18 h of fermentation, respectively. Researchers assumed that possible compounds that have antifungal activity derives from organic acids such as acetic and lactic acids (Yuniarto et al., 2016). In another study to determine the groups of substances that cause antimicrobial effect, order of antimicrobial potency on Salmonella typhimurium (ATCC 14028) was as follows: kombucha (14.0 ± 0.2 mm) obtained from black tea by fermentation for 14 days, kombucha exposed to heat at 120 °C for 15 min (10 ± 0.1 mm), and kombucha neutralized with 1 M NaOH (1.3 ± 0.2 mm) (Al-Mohammadi et al., 2021). Through GC-MS analysis performed to determine the content of kombucha, heterocyclic alcohols, lactones, heterocyclic acids, antibiotics, heterocyclic ester, heterocyclic aldehyde, unsaturated fatty acids, and alkaloids were determined. At the end of this study, the authors concluded that the antimicrobial effect was not only caused by organic acids or heterocyclic acids, but also other substances. Nevertheless, organic acids affect the antimicrobial effect more significantly than proteic structures. Although traditional kombucha is obtained with black tea as a substrate, several studies have been conducted to determine the antimicrobial activity of kombuchas prepared with different substrates. High antimicrobial effect has been observed against bacteria such as Escherichia coli, Shigella dysenteriae, Salmonella typhi, and Vibrio cholera, which are known as intestinal pathogens, in kombucha prepared from black tea, green tea, and oolong tea and in kombuchas obtained as a result of high-temperature denaturation of these teas (Kaewkod et al., 2019).

Antifungal activity of the ethyl acetate fraction of traditional kombucha was studied against a lipophilic fungus, i.e. the genus Malassezia, which plays a role in the development of seborrheic dermatitis (Mazmoudi et al., 2016). For this purpose, 23 clinical isolates of Malassezia ssp., were identified in 19 patients with seborrheic dermatitis and used in the study. Antifungal activity of the ethyl acetate fraction of kombucha was studied at concentrations of 10, 40, and 80 mg/mL and found to display a concentration-dependent inhibition. MIC of ketoconazole against M. furfur, which is the most abundant in clinical isolates from seborrheic dermatitis cases, was 0.6 ± 0.02 μg/mL, while MIC of the ethyl acetate fraction of kombucha was 1.5 ± 0.3 mg/mL. Antifungal activity of kombucha at 80 mg/mL and the antifungal activity of ketoconazole at 4.8 μg/mL was found to be equal to each other. As aforementioned, acetic acid, which is the most abundant organic acid found in kombucha, has always been valued for its antimicrobial activity. It is also one of the components that provide the unique sour taste of kombucha. The mechanism of action is not fully known. Although the antimicrobial effect decreases at high temperatures, it has been again understood that not only phenolics, but also proteic substances have been claimed to contribute to the antimicrobial effect (Kaewkod et al., 2019). The reason for the antimicrobial effect of kombucha could be caused by the metabolites produced by the metabolism of yeast and bacteria in SCOBY.

Another study was conducted to investigate the effects of the degree and duration of the fermentation, pH, and glucuronic acid in amount of kombucha (Ansari et al., 2019). Tea fermented at 20 °C was found to become more acidic. However, according to the results of the analysis made by HPLC, tea fermented at 30 °C contains twice more glucuronic acid than the tea fermented at 20 °C. S. aureus, S. typhimurium, and Lactobacillus rhamnosus strains were used to determine the antibacterial activity of these teas. Antibacterial activity was determined using agar well and disk diffusion assays. Unfermented black tea was used as control group, which exhibited no antibacterial activity against any of these strains. As a result of the analyses performed by both methods, neither kombucha nor gentamicin (10 IU), which was used as positive control, had antibacterial activity against L. rhamnosus. The highest antibacterial activity against S. aureus was observed with kombucha fermented at 20 °C for 21 days (14.3 ± 1.1 mm by agar disk method and 20.0 ± 0.0 mm by agar well method). The most effective kombucha against S. typhimurium was the one fermented at 30 °C for 21 days (16.3 ± 0.6 mm by agar disk method and 22.3 ± 0.6 mm by agar well method). It is put forward that the acetic acid in kombucha causes acidification of the cytoplasm of the bacteria, which stops cell growth and kills the bacteria (Kaewkod et al., 2019).

3.4. Antihypertensive activity

Angiotensin-converting enzyme (ACE) inhibitors such as captopril are a group of drugs used to treat high blood pressure through inhibiting the formation of angiotensin II (Messerli et al., 2018). In a study conducted to determine the effect of fermentation time on ACE inhibition,
Table 1
Antimicrobial activity results expressed by inhibition zone diameters (mm) in kombucha samples.

| Reference | Kombucha substrate | Carbon source | Fermentation duration and temperature | SCOBY | Tested microorganism | Inhibition zone diameter (mm) | Method |
|-----------|---------------------|---------------|---------------------------------------|--------|-----------------------|------------------------------|--------|
|           |                     |               |                                       |        | N.S.                  |                              |        |
|           |                     |               |                                       |        | Glucose               |                              |        |
|           |                     |               |                                       |        | P. acidilactici       |                              |        |
|           |                     |               |                                       |        | A. aceti              |                              |        |
|           |                     |               |                                       |        | Lactobacillus        |                              |        |
|           |                     |               |                                       |        | L. acidophilus        |                              |        |
|           |                     |               |                                       |        | A. pasteurian         |                              |        |
|           |                     |               |                                       |        | A. aceti              |                              |        |
|           |                     |               |                                       |        | A. aceti              |                              |        |
|           |                     |               |                                       |        | A. aceti              |                              |        |
|           |                     |               |                                       |        | A. aceti              |                              |        |

(continued on next page)
Table 1 (continued)

| Reference Kombucha substrate | Carbon source | Fermentation duration and temperature | SCOBY Tested microorganism | Inhibition zone diameter (mm) | Method |
|-------------------------------|---------------|---------------------------------------|----------------------------|-------------------------------|--------|
| (Kaewkod et al., 2019)        | Black tea    | 15 days Room temperature               | S. dysenteriae             | 21.0 ± 0.0 (Black tea)         |        |
|                               | Oolong tea   |                                       |                            | 19.3 ± 0.6 (Oolong tea)        |        |
|                               | Green tea    |                                       |                            | 21.7 ± 0.6 (Green tea)         |        |
|                               |              |                                       |                            | 21.0 ± 0.0 (Black tea)         |        |
|                               |              |                                       |                            | 23.7 ± 0.6 (Oolong tea)        |        |
|                               |              |                                       |                            | 24.7 ± 0.6 (Green tea)         |        |

Vibrio cholera

| Reference Kombucha substrate | Carbon source | Fermentation time and temperature | SCOBY Tested microorganism | Inhibition zone diameter (mm) | Method |
|-------------------------------|---------------|-----------------------------------|----------------------------|-------------------------------|--------|
| (Ansari et al., 2019)         | Black tea    | 21 days 20 °C                     | S. aureus (PTCC 1112)     | 14.3 ± 1.1                    | Disk method |
|                               | Sucrose       |                                    | S. typhimurium (PTCC 1709) | 16.3 ± 1.1                    |        |
|                               |               |                                    | L. rhamnosus (PTCC 1637)   | N.A.                          |        |
|                               |              |                                    |                            | 12.3 ± 0.6                    | Disk method |
|                               |              |                                    |                            |                                |        |
|                               | Black tea    | 21 days 30 °C                     | S. aureus (PTCC 1112)     | 20.0 ± 0.0                    | Agar well diffusion method |
|                               | Sucrose       |                                    | S. typhimurium (PTCC 1709) | 20.3 ± 2.1                    |        |
|                               |               |                                    | L. rhamnosus (PTCC 1637)   | N.A.                          |        |
|                               |               |                                    |                            | 22.3 ± 0.6                    |        |


| Table 2 Antimicrobial activity results expressed by inhibition zone minimum inhibitory concentration in kombucha samples. |
|------------------------------------------------------------------------------------------------------------------|
| Reference Kombucha substrate | Carbon source | Fermentation time and temperature | SCOBY Tested microorganism | MIC (Minimum inhibitory concentration) mean ± standard deviation | Method |
|-------------------------------|---------------|-----------------------------------|----------------------------|---------------------------------------------------------------|--------|
| (Mahmoudi et al., 2016)       | Black tea    | 14 days 24 °C                     | N.S.³                      | 1.5 ± 0.3                                                     | Broth microdilution method |
|                               | Sucrose       |                                    | Malassezia furfur (Clinical isolate) | 1.2 ± 0.2                                                      |        |
|                               |               |                                    | Malassezia globosa (Clinical isolate) | 1.08 ± 0.2                                                     |        |
|                               |               |                                    | Malassezia slooffie (Clinical isolate) | 1.1 ± 0.05                                                     |        |
|                               |               |                                    | Malassezia symphogelous (Clinical isolate) | 0.9 ± 0.08                                                     |        |


³ No activity revealed.

⁴ Not specified.

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kombucha was prepared from *E. camaldulensis* and *Litsea glaucescens* Kunth infusions (Gamboa-Gomez et al., 2016). *E. camaldulensis* (1.40 ± 0.05 μg/µL) and *L. glaucescens* (0.70 ± 0.02 μg/µL) were found to be quite effective, when the IC50 values of ACE inhibition by the plant infusions were compared with captopril (2.75 ± 0.15 μg/µL), which was used as the reference. After fermentation, ACE inhibitory activity decreased, but still *E. camaldulensis* (2.61 ± 0.01 μg/µL) and *L. glaucescens* (1.08 ± 0.01 μg/µL) exerted higher inhibition than that of the reference, considering their IC50 values. The decrease in ACE inhibition may be related to the decreased total phenol and flavonoid contents following fermentation.

### 3.5. Anti-inflammatory activity

In a study carried out by Villarreal-Soto et al. (2019), the ethyl acetate, butanol, and water fractions of tea prepared by fermentation with sugary black tea and kombucha consortium were processed with solvents of increasing polarity. The ethyl acetate fraction of unfermented tea at a concentration of 50 μg/mL inhibited 5-lipoxygenase (LOX) by 66.0 ± 1.7% (IC50 >50 μg/mL), whereas the butanol and aqueous fractions did not inhibit the enzyme. The ethyl acetate, butanol, and aqueous fractions of kombucha obtained as a result of fermentation inhibited the enzyme by 87.7 ± 0.4% (IC50 = 24.3 ± 0.2 μg/mL), 36.9 ± 0.5% (IC50 >50 μg/mL), and 19.0 ± 0.9% (IC50 >50 μg/mL), respectively. It was determined that the components responsible for the anti-inflammatory effect were more present in the ethyl acetate fraction. In another study, Wang et al. (2021) studied anti-inflammatory activity of kombucha in C57BL/6 mice with lipopolysaccharide (LPS)-induced sepsis. It has been shown that orally-administered kombucha reduced the levels of tumor necrosis factor-α (TNF-α) and interleukins (IL-1) and IL-6, which are cytokines formed in response to inflammation.

### 3.6. Hepatoprotective activity

Non-alcoholic fatty liver disease (NAFLD), characterized by macrovesicular steatosis of the liver, is the first common disease among liver diseases (Hyun et al., 2016). In a study on Db/db mice fed a methionine/ choline-deficient (MCD) diet, kombucha was given orally for 3 weeks to mice (Hyun et al., 2016). Aspartate transaminase (AST), alanine aminotransferase (ALT), and triglyceride (TG) levels were found to be lower in mice supplemented with kombucha compared to mice fed with MCD diet and water. In addition, as a result of histological studies, it has been shown that kombucha accelerates the regeneration of the liver. During hepatotoxicity, serum aminotransferases such as ALT, AST, and bilirubin levels increase.

### 3.7. Anticancer activity

Numerous *in vitro* and *in vivo* studies have shown that kombucha has varying levels of anticancer effect or potentiates the effect alone or when administered in combination with currently used anticancer agents. In a study conducted in HCT 116 cell line (human colon cancer), the effect of fermentation time on apoptosis, cell cycle, and cytotoxicity of green tea-derived kombucha was investigated using the MTT assay (Raoouli et al., 2021). Cytotoxic effect of kombucha increased approximately 1.5-fold, when combined with doxorubicin, a clinically used anticancer agent. In cell cycle analysis, the number of cells in the G0/G1 phase increased, while cells in the S phase decreased, which pointed out to intensification in the induction of apoptosis in the early phase. PCR analysis revealed that the expression of Bax, p-53, and p21 genes, which accelerate the progression of apoptosis, also increased. A study was conducted to investigate the effects of different substrates, temperatures, and pH on cytotoxicity during the preparation of kombucha. The cytotoxic effect of kombucha prepared by 15-day fermentation based on green tea, oolong tea, and black tea was evaluated using colorectal cancer cell line (Caco-2), whereas cytotoxicity to the non-cancer cells of the mouse NIH3T3 embryonic fibroblast cell line was also determined (Kaewkod et al., 2019). Although cytotoxicity of each sample was higher in cancer cell lines, it was also observed to be cytotoxic to normal cells. The cytotoxicity amplified with the fermentation of the tea samples. When pH of the obtained kombuchas was adjusted to pH: 7 with 1 M NaOH, the cytotoxicity decreased significantly. The organic acids in kombucha seem to play a role in cytotoxicity. As with antimicrobial activity, an acetic acid solution was prepared at the same pH for determining to what extent acetic acid in kombuchas may play a role in anticancer activity. When applied to the same cell lines, the IC50 values (expressed as % in this study) increased pointedly. While IC50 value of green tea-substrated kombucha in Caco-2 cell line was 2.603 ± 0.072%, IC50 of the acetic acid solution at the same pH was determined to be 33.155 ± 2.834%. The kombuchas were exposed to 121.5 °C for 15 min to denature the proteinaceous substances in their structure. IC50 value (1.309 ± 0.117%) of the kombucha mentioned decreased against the cancer cell line, while the cytotoxic effect increased. It has been shown that the compounds responsible for the anticancer activity are not acetic acid and proteinaceous substances directly, but the substances producing acidity are also responsible for the cytotoxic activity. In a study in which kombucha was prepared using the n-hexane fraction of the fruits of *S. nigrum* as substrate, a significant effect was observed in the study of cytotoxic activity on the MCF-7 breast cancer cell line (IC50: 544.30 ppm) (Ziska & Agustina, 2019). Green tea prepared by the same technique had a lower cytotoxic activity in the same cell line. However, cytotoxic effect of the n-hexane fraction of *S. nigrum* fruit was lower after fermentation (IC50: 1386.39 ppm). To determine the group of compounds responsible for the effect, various flavonoids, saponins, tannins, and steroids were determined through TLC analysis. As a result of the analysis of the hexane fraction by GC–MS, it contains ethylene glycol, 1,3-benzenedicarboxylic acid, 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 9-octadecenetricarboxylic acid, 1,4-benzenedicarboxylic acid, bis(2-hydroxyethyl) ester, 2,6 diamino-4,4′-di-tert-butylbiphenyl, and 7,7-bis(methylthio)-6-methyl-1-(2-thienyl)-2,4,6-heptatrien-1-one was determined. It was commented that these substances may also contribute to anticancer activity of the kombucha.

Studies have been carried out to determine from which substance/s in the content of kombucha caused anticancer activity. In this regard, anticancer activities of polyphenols directly in tea or substances such as organic acids and vitamins formed after fermentation were tested.

### 3.8. Neuroprotective activity

In a study to evaluate the neuroprotective effect of traditional kombucha, a model of cerebral ischemia with middle cerebral artery occlusion was created in Wistar albino rats (Kabiri & Setorki, 2016). Kombucha at doses of 5 and 15 mg/kg was administered intraperitoneally once daily. The results at the end of the experiment showed that kombucha increased the integrity of the blood–brain barrier (BBB) and reduced cerebral edema, infarct volume, and cerebral barrier permeability. At the same time, kombucha inhibited oxidative damage by significantly reducing malondialdehyde (MDA) levels in the brain and plasma. Researchers suggested that kombucha may have a protective effect in ischemia- and reperfusion-induced brain damage.

### 3.9. Wound-healing activity

In a study investigating the wound-healing effect of the ethyl acetate and the water fractions of traditional kombuchas prepared from black tea on Wistar albino rats, an antibacterial 0.2% nitrofurazone ointment was used as positive control (Barati et al., 2016). Histopathological analyzes indicated that kombucha had an effect very close to that of the positive control, accelerating angiogenesis and the formation of connective tissue in the injury area and reducing infection.
3.10. Antidiabetic activity

Antidiabetic effect of kombucha, prepared by 14-day fermentation of the juice from the fruits of Salacca zalacca (Gaertn.) Voss, locally known as “salak suwaru” in Malaysia and “snake fruit” in English, was investigated in streptozocin-induced Wistar albino rats (Zubaidah et al., 2018). Kombucha was administrated orally at doses of 5, 10, and 15 mL/kg for 28 days, and fasting blood glucose was measured every day. Blood analysis disclosed that SOD activity increased, whereas the MDA level diminished. Thus, it is fair to speak of the antioxidant effect of orally consumed kombucha. In rats whose pancreas was isolated for histopathological examination, it was observed that the structure of the islet of Langerhans improved and pancreatic beta cells regenerated compared to the diabetic rat group. The highest therapeutic effect was observed with kombucha at a dose of 5 mL/kg. The substance(s) responsible for the therapeutic effect was not scrutinized. The mechanism of action of the hypoglycemic effect of kombucha and the substance(s) responsible for the effect is not yet fully understood.

3.12. Antihypercholesterolemic activity

Hypercholesterolemia is characterized by low levels of HDLc and high level of LDLc, which increase the risk of cardiovascular diseases such as stroke, myocardial infarction, and atherosclerosis. On the other hand, during hypercholesterolemia, deterioration of liver and kidney functions may be observed. As ROS levels increase, oxidation of LDLc upsurges leading to vascular lesions (Mohamed et al., 2017). In a study to investigate hypcholesterolemic effect of kombucha, Wistar rats were fed on a high-cholesterol diet to induce hypercholesterolemia. Kombuca prepared by fermentation of green tea along with green tea itself was orally administered to the rats at a dose of 5 mL/kg green per day for 4 months. Although both samples improved plasma lipid levels at the end of the experiment, the highest efficacy was observed with kombucha, where it led to a decrease in TG, LDLc, and VLDLc, while HDL levels increased. Besides the levels of SOD and CAT in the liver and kidneys increased and antioxidant activity was enhanced, which led to prevention of oxidation of and delay of inflammation. It has also been suggested that the possible antihypercholesterolemic effect of kombucha could be due to the acetic acid in it. This is because acetic acid activates AMP-activated protein kinase (AMPK), which inhibits fatty acid synthesis and reduces the amount of malonyl-CoA in the liver. In an experiment conducted on 4 groups of albino dwarf rats, the first group of mice was fed with a normal diet, while the second group, in addition to the normal diet, was fed with kombucha made from black tea orally administered at a dose of 2.5 mL/kg once daily. A high-fat diet was applied to the third group, while the fourth group was given kombucha made from black tea at a dose of 2.5 mL/kg orally once a day in addition to the high-fat diet. The outcomes pointed out to an increase in HDL and decrease in LDL levels in the 2.5 mL/kg orally administered group of kombucha. In addition, homocysteine levels, which increased with high-fat diets, were lowered by kombucha. Actually, a high homocysteine level is well-known to be the precursor of many cardiovascular diseases as well as the cause of diseases such as osteoporosis, Alzheimer’s and Parkinson’s diseases, and stroke. In contrast to all of mentioned studies, a study conducted on albino dwarf rats revealed that the use of kombucha and green banana flour as prebiotics in a carbohydrate- and fat-based diet that has become popular in Western culture did not regulate impaired lipid levels and liver function (Urrutia et al., 2021). They showed a very weak effect on liver function markers, i.e. ALT and AST. Despite its high polyphenol content, kombucha showed a weak antioxidant activity. Less weight gain was observed in the group taking kombucha and green banana flour together.

4. Conclusion

Kombucha has become a favorable beverage more recently despite of its ancient past originating from China. It is conventionally prepared with black tea or green tea with addition of sugar, which leads to formation of a biofilm (SCOBY), a bacterial cellulosic structure. The studies indicated that chemical composition of kombucha may vary according to substrate used. However, since black tea is the most widely used one, kombucha mainly contains phenolic acids, organic acids, tea catechins, and other polyphenols, i.e. flavonoids. Not only type of substrate used, but also other factors such as duration of fermentation as well as fermentation conditions, e.g. pH, temperature, sugar concentration, fermentation vessel and volume, etc. have critical role in compositional formation of kombucha. It seems like longer fermentation period may improve the biological activities of kombucha as mentioned in the current review. In addition to strong antioxidant and antimicrobial effects described for kombuchas, anti-aging, anti-hypertensive, anti-inflammatory, hepatoprotective, anticancer/cytotoxic, neuroprotective, wound healing, anti-diabetic, and anti-hypercholesterolemic activities were attributed to kombucha, which make it a healthy popular drink. Although kombucha has many desired effects for human health as aforementioned, the experimental analyses to identify its chemical composition are very important because standardization of kombucha will be expedited by defining the group of bioactive substances responsible for the aforementioned pharmacological effects. Consequently, more advanced chemical and bioactivity studies on kombucha should be elaborated.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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