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Short communication

Turmeric Kombucha as effective immunomodulator in *Salmonella typhi*-infected experimental animals

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ABSTRACT

This study investigated the immunomodulatory effects of turmeric and black tea kombucha in the experimental animal. The kombucha was administrated orally to non-infected as well as *Salmonella typhi*-infected Balb-C mice and samples were taken to study the *in vivo* immunomodulatory activity of the kombucha. Both turmeric and black tea kombucha enhanced the adaptive immune response (indicated by an increase in $CD4^+$, TNFα, IFN-γ) and innate immune response (evidenced by a decrease of CD68 IL-6). Although both samples showed notable positive results, the *in vivo* immunomodulatory activity of turmeric kombucha was much pronounced compared to black tea kombucha.

1. Introduction

Bacterial infections can harm human body and cause diseases. Fortunately, vigilant immune system plays a pivotal role against pathogenic bacteria and foreign objects. Supported with immunomodulators, the immune system is constantly enhanced by either immune-stimulants or immune-suppressants (the latter suppress abnormal reactions) (Chaplin, 2010). Kombucha has been reported for its effective immunomodulatory properties (Zubaidah et al., 2021). Kombucha is a beverage made from sweetened tea infusion which is then fermented by a consortium of symbiotic culture of bacteria and yeast (SCOBY) (Kapp et al., 2018). Various yeasts (e.g. *Pichia, Candida, Zygosacharomyces, Brettanomyces,* and *Saccharomyces*) and acetic acid bacteria (e.g. *A. xylinum*) have been identified in kombucha (Jayabalan et al., 2014).

Kombucha can be made from tea and or other potential ingredients (e.g. Turmeric). Turmeric (*Curcuma longa*) is a medicinal herb that contains bioactive compounds such as: polyphenol and derivatives of curcuminoids (demethoxycurcumin and bidemetoxycurcumin), the literature reported that these curcuminoids have antioxidants, anti-inflammatory, and anti-cancer properties (Karimian et al., 2017). Curcuminoids, which are derivatives of curcumin, have antioxidant, anti-inflammatory, and immune-enhancing activity (Dosoky and Setzer, 2018). According to Yong et al. (2019), the fermentation of turmeric with *Lactobacillus* increases anti-inflammatory and antioxidant activity, evidenced by suppressing the production of TNF-α and NO (nitric oxide), JNK signaling pathways, and lowering DPPH radicals. Notwithstanding, a study on turmeric kombucha that highlights its immunomodulator has never been reported. Based on recent literature, the bioactive characteristics of black tea kombucha has been explored due to its flavonoid and tannins content which can increase $CD4^+$, $CD8^+$, CD4 + IFNɣ, CD4 + TNFα, $CD8^+$ IFNɣ, and CD8 + TNFα cells in *Salmonella typhi*

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Table 1
Kombucha quality.

Parameter	Turmeric kombucha		Black tea kombucha	
	Day-0	Day-14	Day-0	Day-14
Total microbes (CFU/ml)	1.6×10^2	2.0×10^7	1.2×10^2	1.3×10^8
Titratable acidity (%)	0.15 ± 0.03	0.22 ± 0.00	0.19 ± 0.00	0.58 ± 0.00
pH	4.03 ± 0.06	3.02 ± 0.02	3.96 ± 0.02	2.81 ± 0.08
Total phenolic content (μg GAE/ml)	81.55 ± 8.06	132.89 ± 14.59	386.07 ± 25.08	716.02 ± 85.81
Total sugar (%)	8.95 ± 0.36	7.91 ± 0.15	9.22 ± 0.77	7.83 ± 0.11

Table 2
GC analysis of turmeric kombucha.

Peak	Retention time (minutes)	Compound name	Chemical formula	Molecular weight (g/mol)	Area (%)
3	1.59	Ethanol	C_2H_6O	46	2.50
4	2.20	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-	$C_8H_9N_3O_4$	211	0.08
5	8.50	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	$C_{10}H_{16}$	136	0.28

infected mice (Zubaidah et al., 2021). Hence, this study aimed to investigate and compare the immunomodulatory activities of turmeric versus black tea kombucha.

2. Materials and methods

2.1. Materials

Turmeric (*Curcuma longa*) was obtained from a traditional market in Malang, East Java, Indonesia. Commercial kombucha starter, black tea and cane sugar was purchased from local distributors. All chemicals used were analytical grade purchased from local distributors.

2.2. Kombucha fermentation and analysis

Washed turmeric was cut into small pieces, dried with a cabinet dryer (60 °C, 5 h), ground into a powder, and put in a teabag. Six (6)g turmeric powder was extracted in 500 ml of boiling water for 5 min and sweetening with 10% sugar. Black tea extraction was conducted according to Ardheniati et al. (2009). The black tea extract was prepared by infusing 2 g black tea in 500 ml boiling water for 5 min and adding 10% sugar. The sweetened turmeric and black tea extracts were put into separate sterile jars, cooled at room temperature before adding kombucha starter (10%). Each kombucha was incubated for 14 days at room temperature. Samples of the fermenting broth were taken at day 0 and day 14 for laboratory analysis (Zubaidah et al., 2018).

The analytical procedures included: Total microbes, titratable acidity, pH, total phenolic content, and total sugar. According to Fardiaz (1987), the total microbial analysis was measured by counting the colonies grow on PCA agar. Titratable acidity was determined using direct titration with NaOH solution [0.1 N] and expressed as % titratable acidity (Apriyantono et al., 1989). pH was measured using a pH meter (Hanna HI-2202 Edge ®blu). Total phenolic content was determined according to Sharma et al. (2011) with measurement of the complex compound formed after reaction with Folin-Ciocalteau reagent and Na_2CO_3 solution, spectrophotometrically at 500–600 nm. Total sugar was determined according to Apriyantono et al. (1989) with Anthrone reagent and H_2SO_4, spectrophotometrically at 600–700 nm.

The GC-MS (Gas Chromatography – Mass Spectrometry) instrument used an HP-5MS UI capillary column with a length of 30 m, a diameter of 250 μm, and a film thickness of 0.25 μm. The carrier gas is helium with a flow rate of 20 ml/min. The initial temperature is 200 °C, running time 15 min, the detection was facilitated by Chromeleon Chromatography Data System (CDS) software.

The immunomodulatory activity assays of turmeric and black tea kombucha were performed *in vivo* using 30 female, six-week-old Balb/c mice, body weight ranged 18–20 grams. This animal study was approved by the Brawijaya University Research Ethics Committee (Ethical Clearance No. KEP-063-UB). Mice were divided randomly into six groups- (P0) is negative control which refers to normal health mice (untreated). (P1) healthy mice + turmeric kombucha, (P2) healthy mice + black tea kombucha, (P3) positive control which refers to mice that infected by *Salmonella typhi*, (P4) infected mice + turmeric kombucha, (P5) infected mice + black tea kombucha. Kombucha was administered orally at a dose of 0.31 ml/kg/BW/day for 21 days. *Salmonella typhi* 10^8 CFU/ml was injected into mice on the 22nd day then incubated for seven days. CD4 +, TNF-α, IFN-γ, CD68, and IL-6 were analyzed using flow cytometry (Zubaidah et al., 2021).

2.3. Statistical analyses

Analysis of Variance (ANOVA) was employed to analyse the data and further statistical tests such as the Fisher method at $\alpha = 5\%$ has been applied.

Table 3
Adaptive Immunomodulatory activity of kombucha.

Group	$CD4^+$ (%)	$CD4^+$ TNF-α^+ (%)	$CD4^+$ IFN-γ^+(%)
P0 (Negative control)	$3.55^{c} \pm 1.03$	$0.60^{b} \pm 0.10$	$1.21^{b} \pm 0.26$
P1 (Healthy mice + turmeric kombucha)	$5.22^{b} \pm 0.60$	$0.85^{ab} \pm 0.14$	$1.28^{b} \pm 0.31$
P2 (Healthy mice + black tea kombucha)	$6.00^{b} \pm 0.71$	$0.93^{ab} \pm 0.43$	$1.37^{ab} \pm 0.27$
P3 (Positive control)	$5.09^{bc} \pm 0.63$	$0.84^{ab} \pm 0.13$	$1.46^{ab} \pm 0.21$
P4 (Infected mice + turmeric kombucha)	$8.13^{a} \pm 1.00$	$1.41^{a} \pm 0.63$	$1.79^{a} \pm 0.26$
P5 (Infected mice + black tea kombucha)	$6.61^{ab} \pm 1.10$	$1.12^{ab} \pm 0.19$	$1.64^{ab} \pm 0.18$

Values are means ± standard deviations (n = 4). Different letter in the same column mean significant different at $\alpha = 5\%$ ($p < 0.05$).

3. Results and discussion

Table 1 presents the characteristics of turmeric and black tea kombucha. On day-14, black tea kombucha recorded 1.3×10^8 CFU/ml total microbes, higher than turmeric kombucha 2.0×10^7 CFU/ml. The GC analysis (Table 2) of turmeric kombucha showed the presence of compounds Butanenitrile, 2,3-dioxo-, dioxime, O, O'-diacetyl- ($C_8H_9N_3O_4$) and Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S) - ($C_{10}H_{16}$). Several studies have shown the involvement of nicorandil ($C_8H_9N_3O_4$) in the inflammatory process and oxidative stress regulation (Ashour et al., 2020). The analysis of immune response in this study is presented in Table 3. It worth highlighting that the effect of kombucha's immunomodulatory activity showed a significant difference ($P < 0.05$) between the treatment group and the control group. Immune response analysis also carried out on the innate immunity with macrophage $CD68^+$ IL-6^+ (Fig. 1). The statistical results showed significant differences ($p < 0.05$) between the treatment and control groups. The reduction of $CD68^+$ IL-6^+ might be due to bioactive compounds in kombucha that stimulate innate response when infected with *Salmonella typhi,* macrophage phagocyte pathogenic bacteria and normalizing the infected immune system. Phenolic and curcumin content in turmeric can block the NF-kB and TLR2 pathways, regulating inflammatory response that causes cell apoptosis and inhibit IL-6 expression (Rocha-Ramírez et al., 2017; Liu et al., 2006). Despite flavonoids have been shown to increase IL-2 and lymphocyte proliferation (Nopitasari, 2006), it worth noting that bioactive compounds and the symbiosis of bacteria and yeasts can enhance immunity and act as antimicrobials (Watawana et al., 2015). Hence, based on previous study, black tea kombucha was qualified to be administrate as immunomodulatory agent for *S. typhi* infected mice due to its beneficial effect that increases $CD4^+$, $CD8^+$, CD4 + IFNɣ, CD4 + TNFα, $CD8^+$ IFNɣ, and CD8 + TNFα cells (Zubaidah et al., 2021). This property is mirroring its notable contents of phenolic compound and organic acid present in black tea kombucha. In the same way, curcumin is one of the bioactive compounds in turmeric, which has antimicrobial activity (Shlar et al., 2017). Moreover, curcumin also contains curcuminoids, demethoxycurcumin, bisdemethoxycurcumin, dihydrocurcumin, xanthorrhizol, turmeronol, curdione, curcuzedoalide, curcumenol, and germacrone which display significant effect on immune system (Yuandani et al., 2021). These compounds in turmeric may enhance the immunomodulatory activity in turmeric kombucha. Thus, the *in vivo* immunomodulatory activity of turmeric kombucha ($CD68^+$ IL-6^+ macrophage on *S. typhi* infected mice) was much higher when compared to those demonstrated in black tea kombucha.

4. Conclusions

Our finding highlighted that turmeric kombucha and black tea kombucha enhance the adaptive immune response (evidence by an increase in $CD4^+$, TNFα, IFN-γ) and innate immune response (denoted by a decrease of CD68 IL-6). However, the *in vivo* immunomodulatory activity of turmeric kombucha was much higher than black tea kombucha.

Declaration of competing interest

We declare that there are no conflict of interest.

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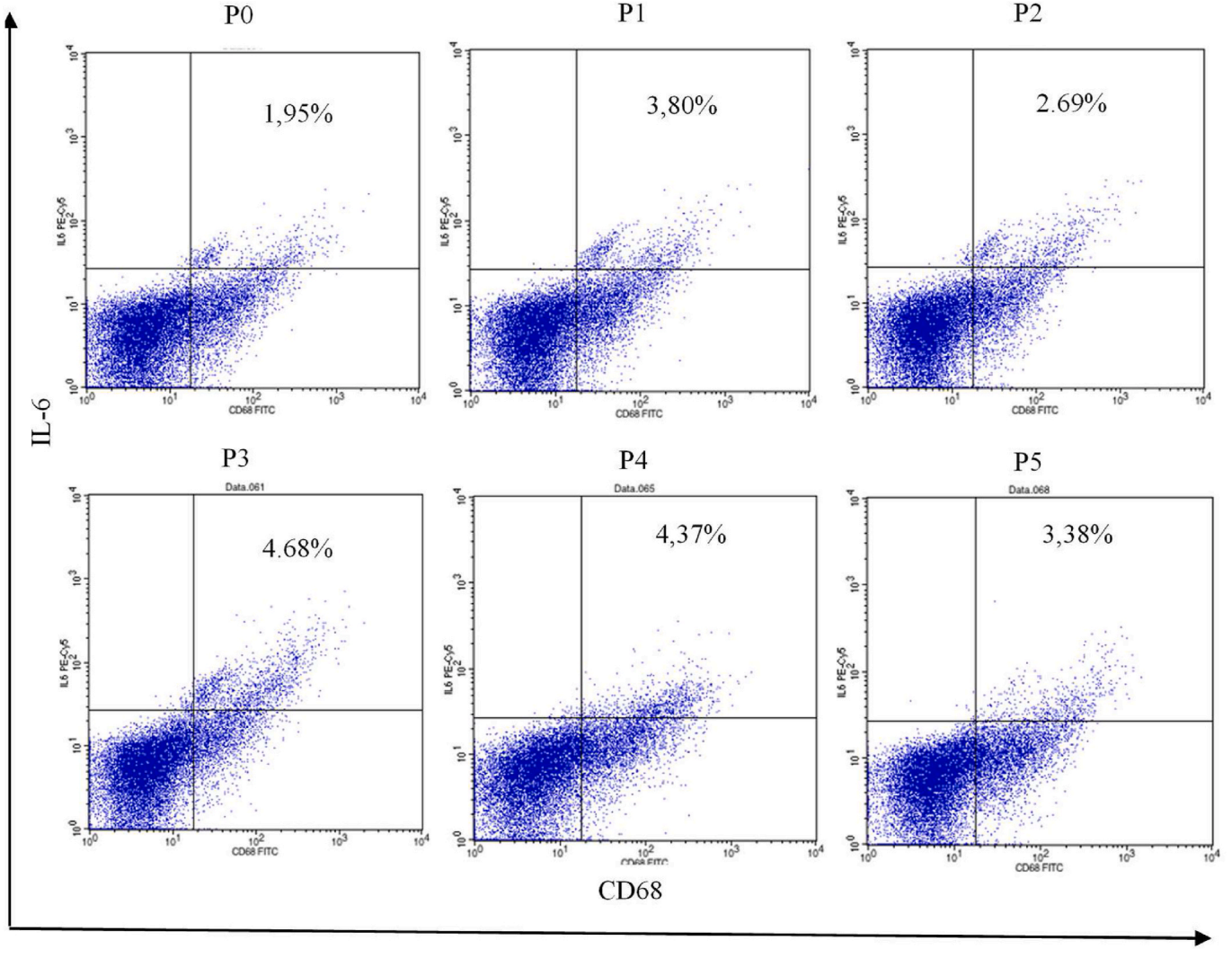

Fig. 1. Innate immunomodulatory activity of kombucha: $CD68^{+}$ $IL\text{-}6^{+}$ macrophage at different treatments: (P0) negative control. (P1) healthy mice + turmeric kombucha, (P2) healthy mice + black tea kombucha, (P3) positive control, (P4) infected mice + turmeric kombucha, (P5) infected mice + black tea kombucha.

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