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RESEARCH ARTICLE

Physicochemical Properties and Anti-cancer Activity of Javanese Turmeric Kombucha (*Curcuma xanthorrhiza*) Against T47D Cell Line

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Abstract

Breast cancer constitutes a growing global health crisis, with rising incidence and mortality alongside therapy-limiting adverse effects. Kombucha, a fermented beverage made from tea using a symbiotic culture of bacteria and yeast (SCOBY), has demonstrated various health-promoting properties. This study explores a novel substrate, Javanese turmeric (*C. xanthorrhiza*), an indigenous Indonesian herb known for its hepatoprotective, antioxidant, antidiabetic, and antimicrobial properties. The anti-cancer potential of Javanese turmeric kombucha (JTK) was evaluated as a complementary therapy against T47D cells by comparing its physicochemical and antioxidant properties with those of unfermented Javanese turmeric beverage (JTB), and assessing its cytotoxicity, morphological effects, and ability to induce apoptosis. Data were analyzed using analysis of variance (ANOVA), followed by post hoc tests such as Fisher's method ($\alpha = 0.05$). Statistical analyses were conducted using Minitab 17.0 programs. The results showed that JTK exhibited significantly higher levels of total phenols (162.61 ± 0.32 mgGAE/mL) and antioxidant activity (IC50 157.07 ± 2.75 ppm) compared to unfermented JTB. JTK also induced apoptosis in more than 22% of the T47D cell population, indicating a promising cytotoxic potential. The findings highlight that fermentation enhances the physicochemical characteristics and anti-cancer properties of Javanese turmeric, suggesting that kombucha derived from this plant may serve as a promising complementary beverage in breast cancer therapy.

Keywords: Kombucha, Fermentation, Javanese turmeric, Anti-cancer, T47D cells

Introduction

Breast cancer ranks as the most prevalent malignancy and leading cause of cancer mortality in women globally [1], where conventional therapies (chemotherapy, radiotherapy, surgery) are limited by organ toxicity, acquired resistance, and poor prognosis [2]. Natural compounds offer promise as complementary agents by enhancing chemosensitivity, reducing side effects, and targeting multiple oncogenic pathways. Many medicinal plants contain bioactive phytochemicals that exhibit both chemopreventive and chemopotentiation activities. A promising example is kombucha, a traditionally fermented beverage produced by symbiotic cultures of bacteria and yeast (SCOBY).

Kombucha is a non-alcoholic or low-alcohol fermented beverage with functional effects on the body. Bioactive compounds identified in kombucha tea include phenolic compounds, flavonoids, vitamins, amino acids, and organic acids such as gluconic acid, glucuronic acid, acetic acid, ascorbic acid, succinic acid, and D-saccharic acid 1,4-lactone [3]. Consumption of kombucha has health benefits including preventing neurodegenerative diseases, lowering blood pressure, antioxidant activity, hypoglycemic effects, detoxification activity, and anti-cancer properties attributed to metabolites produced during fermentation [4].

Even though tea is the traditional substrate to prepare kombucha, other natural ingredients, including herbs, are being explored as substrates to improve selected biological functions and enhance the beverage's flavour. Recent studies on rhizome-based fermentation highlighted the increased content of bioactive compounds that have various metabolic functions, including antioxidant activity in ginger kombucha [5]; immunomodulatory, hepatoprotective effects of turmeric kombucha [6]; and immunomodulatory effect of Javanese turmeric [7]. These studies show that fermented rhizomes have better functional properties than unfermented rhizome beverages.

Javanese turmeric is a native Indonesian rhizome traditionally used in herbal medicine due to its therapeutic properties [8]. This plant contains bioactive compounds such as: Terpenoids, flavonoids, curcuminoids, and non-curcuminoid components such as ar-turmerone, α-turmerone, curcumene, bisacurone, curlone, lactone-germacrone, and germacrone [9]. The profile of essential bioactive compounds in Javanese turmeric has been shown several health advantages including antioxidants [10,11]; preventing cardiovascular [12]; hepatoprotective [13]; and as an anti-cancer [14,15]. Kombucha contains a variety of bioactive compounds, including polyphenols, organic acids, vitamins, and other metabolites. The synergistic effects of these compounds can enhance their anti-cancer properties [16].

Although kombucha has been consumed and investigated for a long time, no research has reported on the anti-cancer activity of Javanese turmeric. Therefore, this study aimed to evaluate JTK as a potential complementary therapy for T47D cells by comparing its physicochemical properties and antioxidant activity with unfermented JTB to identify fermentation-enhanced bioactivity, and assessing anti-cancer effects through cytotoxicity assays, morphological analysis, and apoptosis induction.

Materials and methods

Materials

Javanese turmeric was obtained from the "Oro-Oro Dowo" market in Malang, East Java, Indonesia. The turmeric was washed with mineral water (Aqua, Indonesia), peeled with a knife, sliced into 1 - 3 mm thickness, and dried with a dehydrator (Papalolo, 220V - 400V, 800W) at 60 °C for 6 h. Dried Javanese turmeric was crushed with a blender machine (Phillips Blender HR2221/30, China) into powder [6]. The kombucha starter was obtained from a store (Healthy Secret, original black tea kombucha drink), and the black tea (Tong Tji, Indonesia) and cane sugar (Gulaku, Indonesia) were obtained from a supermarket in Malang, East Java, Indonesia.

Reagents and chemicals

The following reagents were used in this study

Ethanol (≥ 99.8%), gallic acid standard, quercetin standard, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate were from Sigma Aldrich Co., Merck (St. Louis, MO, USA), Phenolphthalein indicator, oxalic acid, Folin-Ciocalteau, aluminum chloride, sodium nitrate, sodium hydroxide and trypsin from Merck KGaA (Darmstadt, Germany), Phosphate-buffered saline (PBS) was from WISENT Inc (Quebec, Canada), and also apoptosis Kit Annexin V and PI (Wuhan Elabscience, China).

Preparation of JTK and JTB

The kombucha starter culture was first prepared by steeping 4 g of black tea (Tong Tji, Indonesia) in 1000 mL boiling water for 4 min, followed by the addition of 10% (w/v) sugar. After cooling to room temperature (25 ± 2 °C), the tea was added with 10% (v/v) kombucha starter and fermented for 14 days. For JTK production, Javanese turmeric rhizomes were processed into 1 - 3 mm slices, dried at 60 °C for 6 h, and ground into powder. Five g of this powder in tea bags was steeped in 500 mL boiling water for 4 min, supplemented with 10% (w/v) sugar, and cooled to room temperature. The turmeric infusion was then added with 10 % (v/v) of the prepared kombucha starter and fermented for 8 days at room temperature [17]. Unfermented JTB control was prepared identically but without adding of kombucha starter (no fermentation). All samples were analyzed for physicochemical properties and then stored at 4 °C for further analysis.

Physicochemical analysis of JTK and JTB

The physicochemical components of JTK and JTB were analyzed on days 0 and 8 of fermentation.

The parameters analyzed included total acid content [18], pH [19], total phenolic content (TPC) [20], total flavonoid content (TFC) [19], and antioxidant activity (IC_{50} via DPPH assay) [20]. pH was measured using a digital pH meter (Hanna HI-5222-02, UK), while titratable acidity was determined by direct titration with 0.1 N NaOH and expressed as % acidity. For TPC, samples were reacted with Folin-Ciocalteu reagent and 20% (w/v) Na_2CO_3, and absorbance was measured at 765 nm; results were expressed as mg gallic acid equivalents (GAE)/mL. TFC was quantified using the aluminum chloride colorimetric method, with results calculated as mg quercetin equivalents (QE)/mL. Antioxidant activity was assessed via the DPPH radical scavenging assay, where the IC_{50} (concentration required to inhibit 50% of DPPH radicals) was determined through linear regression of absorbance data at 517 nm.

Cell culture

T47D cells were cultured in complete Roswell Park Memorial Institute medium (RPMI, Gibco) supplemented with 10% fetal bovine serum (FBS) and 40 mg/L gentamicin. Cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere, with medium changes every 24 h. Upon reaching 70 - 80% confluency, cells were harvested by trypsinization for subsequent experiments [21].

Cytotoxicity assay and cell morphology

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (GLPBIO, USA) [22]. Briefly, T47D cells (1×10^4 cells/well) were seeded in 96-well plates (Biologix, USA) and incubated for 24 h at 37 °C. After washing with PBS, cells were treated with JTK and JTB at concentrations of 500 - 1300 µg/mL and incubated for an additional 24 h. CCK-8 reagent (10 µL/well) was added, and plates were incubated for 3 h. Absorbance was measured at 450 nm using a microplate reader, and viability percentages were calculated as: (Absorbance of treated cells/Absorbance of untreated cells×100% [21]. Morphological changes in treated and untreated (control) cells were observed using an inverted microscope (Nikon TS100, Japan) at 10×magnification [14].

Apoptosis assay

Apoptosis in T47D cells was quantitatively assessed using a validated dual-fluorescence approach combining Annexin V-FITC (Invitrogen, USA) and propidium iodide (PI; Sigma-Aldrich, USA) staining followed by flow cytometry analysis.

Cell staining

Analysis of T47D cell apoptosis was assessed using dual fluorescent staining with Annexin V-FITC (Invitrogen, USA) and propidium iodide (PI) (Sigma-Aldrich, USA). Cells were washed with PBS 3 times for 5 min. Next, the cells were washed with 0.1% Triton-X 100 PBS for 5 min. Cells were then incubated with 1 % bovine serum albumin (BSA) for 30 min at room temperature. Then, the BSA solution was discarded and the cells were added to the solution with primary antibodies. Cells were incubated overnight at 4 °C in dark conditions. Cells were then rewashed with PBS 3 times for 5 min, incubated with DAPI 1:1000 for 5 min, rewashed with PBS 3 times for 5 min, added with glycerine and then covered with mounting medium and cover glass. Cells were observed with a fluorescence microscope (Olympus DP73, Japan) [23].

Flow cytometry analysis

The steps for apoptosis testing were carried out according to the procedures in the Kit. T47D cells were grown on 24-well plates and incubated at 37 °C with 5% CO_2. Cells were then treated with JTK and incubated at 37 °C with 5% CO_2. Cells were trypsinized, harvested, resuspended, and washed using PBS. Cells were then centrifuged, supernatant removed, and resuspended with 50 µL Annexin V FITC/PI (1:2; Invitrogen, USA) and incubated for 30 min in the dark and chilled. Subsequently, 400 µL PBS was added and the mixture was analyzed by flow cytometry (BD Biosciences, USA). Data were analyzed with CellQuest software (BD Biosciences) [14].

Statistical analysis

Analysis of Variance (ANOVA) was used to analyze the data and further statistical tests such as Fisher's method at $\alpha = 5\%$ were applied. The analysis used MS Excel and Minitab 17.0 programs.

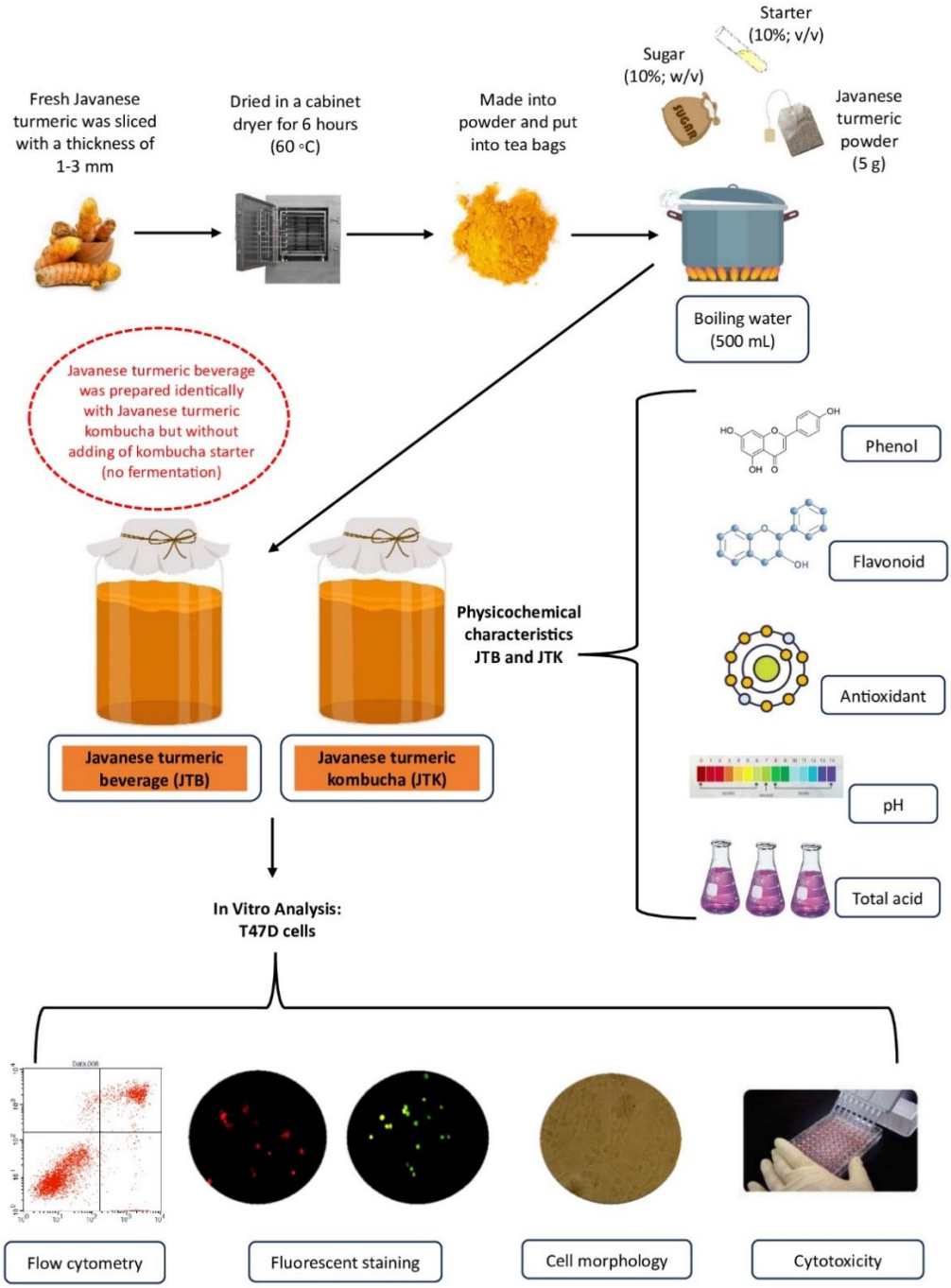

Figure 1 Schematic diagram of the research steps.

Results and discussion

Physicochemical characteristics of JTK

Table 1 presents the physicochemical characteristics of JTK after 8 days of fermentation and compared with JTB. JTK has better physicochemical characteristics than JTB. JTK showed a total acid value of 0.45 ± 0.05%, pH 3.32 ± 0.03, total phenolic content (TPC) 162.61 ± 0.32 mgGAE/mL, total flavonoids content (TFC) 48.99 ± 0.69 mgQE/mL, and antioxidant activity IC_{50} 157.07 ± 2.75 ppm. Kombucha demonstrates superior physicochemical properties compared to the unfermented beverage (**Table 1**), likely due to the enhanced metabolic activity of microorganisms during fermentation. The symbiosis between bacteria and yeast causes the accumulation of organic acids which increase the total acid and decrease the pH of the medium at the end of fermentation [24]. This acidic environment (pH < 4.5) creates functional antimicrobial conditions, making foods with pH 4.0 - 4.5 (acidic) and < 4.0 (highly acidic) microbiologically

safe as they effectively inhibit pathogenic microorganism growth [25].

Previous research on turmeric kombucha showed an increase in total acid after fermentation for 14 days [17]. The total acid value of turmeric kombucha on day 0 was 0.15% then increased to 0.22% on day 14 of fermentation and was associated with a decrease in pH value. The pH value of turmeric kombucha on day '0' of 4.03 decreased to 3.02 on day 14 of fermentation. Another study on kombucha also showed an increase in total acid during 14 days of fermentation where the total acid value on day 0 was 1.60% and on day 14 increased to 4.10% [26].

Table 1 Physicochemical analysis of JTB and JTK.

Parameters	Javanese Turmeric	
	JTB	**JTK**
Total acid (%)	0.14 ± 0.00	0.45 ± 0.05*
pH	3.89 ± 0.03	3.32 ± 0.03***
Total phenolic content (TPC) (mgGAE/mL)	64.09 ± 0.55	162.61 ± 0.32***
Total flavonoids content (TFC) (mgQE/mL)	76.37 ± 1.02	48.99 ± 0.69***
Antioxidant activity IC_{50} (ppm)	264.93 ± 2.90	157.07 ± 2.75***

Data are presented as mean value ± standard deviation (N = 3). Statistical significance: *p < 0.05, **p < 0.01, **p < 0.001. GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent, IC_{50} = Half maximal inhibitory concentration. JTK has better physicochemical characteristics than JTB (without fermentation).

Fermentation in kombucha resulted in an increase in TPC and a decrease in TFC (**Table 1**). This is due to acid hydrolysis and microbial biotransformation of condensed phenolic components. The increase in total phenols in kombucha is also caused by microbial activity in catalyzing the oxidation of complex phenolic compounds into simpler molecules during the fermentation process. These represent the predominant antioxidant constituents, which are largely responsible for its documented health-promoting properties [27]. The same results were shown by previous research on turmeric kombucha. Total phenols increased from 81.55 µgGAE/mL to 132.89 µgGAE/mL after 14 days of fermentation [17].

The decrease in TFC (**Table 1**) is caused by oxidative reactions of polyphenols and flavonoids [28,29]. Some of the complex phenolic compounds present in the raw materials are degraded by enzymes secreted by bacteria and yeast on SCOBY and supported by the acidic environment during fermentation. Lactic acid bacteria that are also found in SCOBY, namely *Lactiplantibacillus plantarum*, have β-glucosidase enzymes that can degrade flavonoids [30]. Previous research on black tea kombucha reported similar findings, showing a significant decrease in TFC from 16.0 ± 0.1 (QE)/(µg/mL) to 13.0 ± 0.2 (QE)/(µg/mL) [31].

The increase in antioxidant activity (**Table 1**) was influenced by kombucha bioactive compounds. The increase in most bioactive compounds at the end of fermentation is caused by biotransformation of primary metabolites that are present in the substrate or due to the breakdown of complex forms of bioactive compounds into simpler forms [32]. This result is in line with previous research on salak kombucha, where the antioxidant activity increased from 79.82% to 89.33% after 14 days of fermentation [33]. Antioxidant activity is related to the content of bioactive compounds in the form of phenolics and flavonoids that can increase radical inhibition due to the capacity of hydroxyl groups to donate their hydrogen atoms to free radicals [34].

Cytotoxicity analysis

Cytotoxicity analysis on JTK compared with JTB are presented in **Table 2**. JTK had the highest decrease in cell viability which reached 14.877% at the highest treatment dose (1300 µg/mL) with the IC_{50} cytotoxicity value is 1.03 mg/mL, while the IC_{50} cytotoxicity value of JTB is 5.89 mg/mL which is 5 times higher than JTK. This refers to the concentration required to induce 50% cell death in T47D cells.

Table 2 Percentage of viability of T47D cells at various doses of treatment.

Doses (µg/mL)	Cell viability (%)	
	JTB	**JTK**
500	99.333 ± 1.13^{a}	98.162 ± 2.02^{a}
700	97.899 ± 1.88^{ab}	94.708 ± 3.70^{a}
900	96.314 ± 2.74^{abc}	71.589 ± 8.30^{b}
1100	94.031 ± 4.66^{bc}	45.066 ± 3.43^{c}
1300	92.092 ± 1.95^{c}	14.877 ± 3.70^{d}
IC_{50} (mg/mL)	5.89	1.03

Data are presented as mean ± standard deviation (N = 4). Values of each sample in the same column with different letters (a - b) differ significantly ($p < 0.05$). IC_{50} cytotoxicity values show that the fermentation process was able to increase the anti-cancer activity of kombucha.

In the cell cytotoxicity analysis (**Table 2**), the fermentation process was able to increase the anti-cancer activity of kombucha by almost 5 times as indicated by the resulting IC_{50} cytotoxicity value. JTK also exhibited superior cytotoxic activity compared to other kombucha formulations tested against cancer cells. The IC_{50} value of JTK on T47D human breast cancer cells was determined to be 1.03 mg/mL, indicating a potent inhibitory effect. This finding suggests that JTK has a higher cytotoxic potency than previously reported kombucha variants. A study evaluating citrus-based kombucha against human bladder cancer cell lines T-24 and 5637 reported higher IC_{50} values of 4 and 7 mg/mL, respectively [35]. The lower IC_{50} value observed for JTK suggests a stronger potential as an anti-cancer agent, particularly in breast cancer therapy.

Fermentation can increase the biological activity and production of bioactive compounds in kombucha which is directly related to its ability as an anti-cancer. Javanese turmeric contains active ingredients that have the potential as anti-cancer, such as keto-curcumin, [13C]-curcumin, piperidine, curcumin, demethoxycurcumin, and xanthorrhizol. Fermentation of JTK for 12 days produces new compounds that also have potential as anti-cancer, including tetrahydrocurcumin (THC)-monoglucuronide, curcumin monoglucoside, curcumin glucuronide [36]. Curcuminoids represent the major class of bioactive compounds in Javanese turmeric. Curcuminoids have been known to have a potent anti-cancer, antioxidant, and antibacterial activity [37].

Cell morphology

T47D cell morphology results based on treatment JTB and JTK were determined and shown in **Figure 2.**

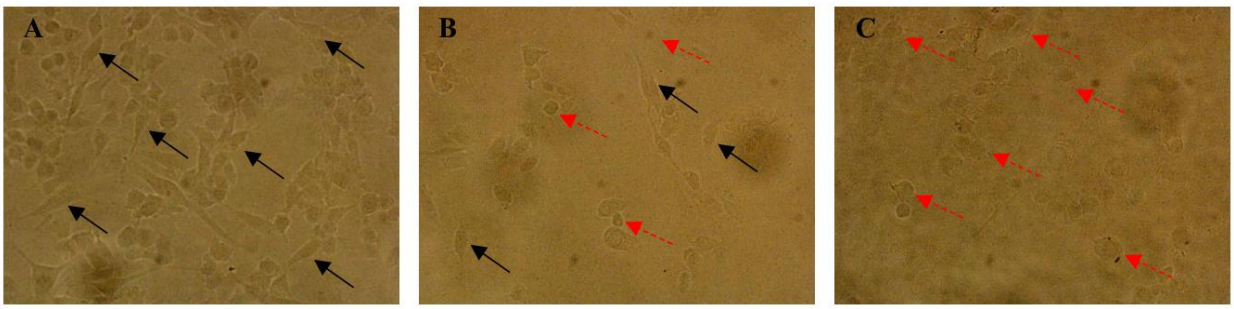

Figure 2 Morphological changes in T47D cells after the treatments. (A) Control: Untreated T47D cells showing normal morphology. (B) JTB-treated cells: Moderate cytotoxic effects observed. (C) JTK-treated cells: Significant increase in cell death compared to JTB and control. Live cells are indicated by black arrows, while dead cells are marked by red dashed arrows. JTK treatment indicated greater T47D cells death compared to JTB.

Based on the microscopic observations in **Figure 2**, there are differences in cell morphology in the control (no treatment) and all treatments. The control showed high cell density with the highest number of live cells. The JTK treatment (**Figure 2(C)**) showed a decrease in both cell density and number of live cells compared to the JTB treatment (**Figure 2(B)**). The appraisal of cell morphology (**Figure 2**) shows differences in each treatment. The morphology of living cells looks shiny and the boundary of the cell membrane with the media is visible. The dead cells appear round and dark and the cell membrane appears broken or slightly faint [38]. Cells that undergo apoptosis are specifically characterized by loss of asymmetry and attachment of the plasma membrane, rupture of the plasma membrane, and condensation of the cytoplasm and nucleus. In addition, cells that experience apoptosis lose their ability to attach to cell culture plates [14]. Observation of the morphology of T47D cells (**Figure 2**) showed that JTK treatment indicated greater T47D cells death compared to JTB treatment. The kombucha fermentation process increases the content of bioactive compounds contained in the ingredients that have the potential to be anti-cancer. In JTK, fermentation increased the content of bioactive compounds in the form of xanthorrhizol. Xanthorrhizol has apoptosis induction activity by regulating p53 in the mitochondrial pathway of cancer cells with diverse regulations on Bax/Bcl-2 expression [39].

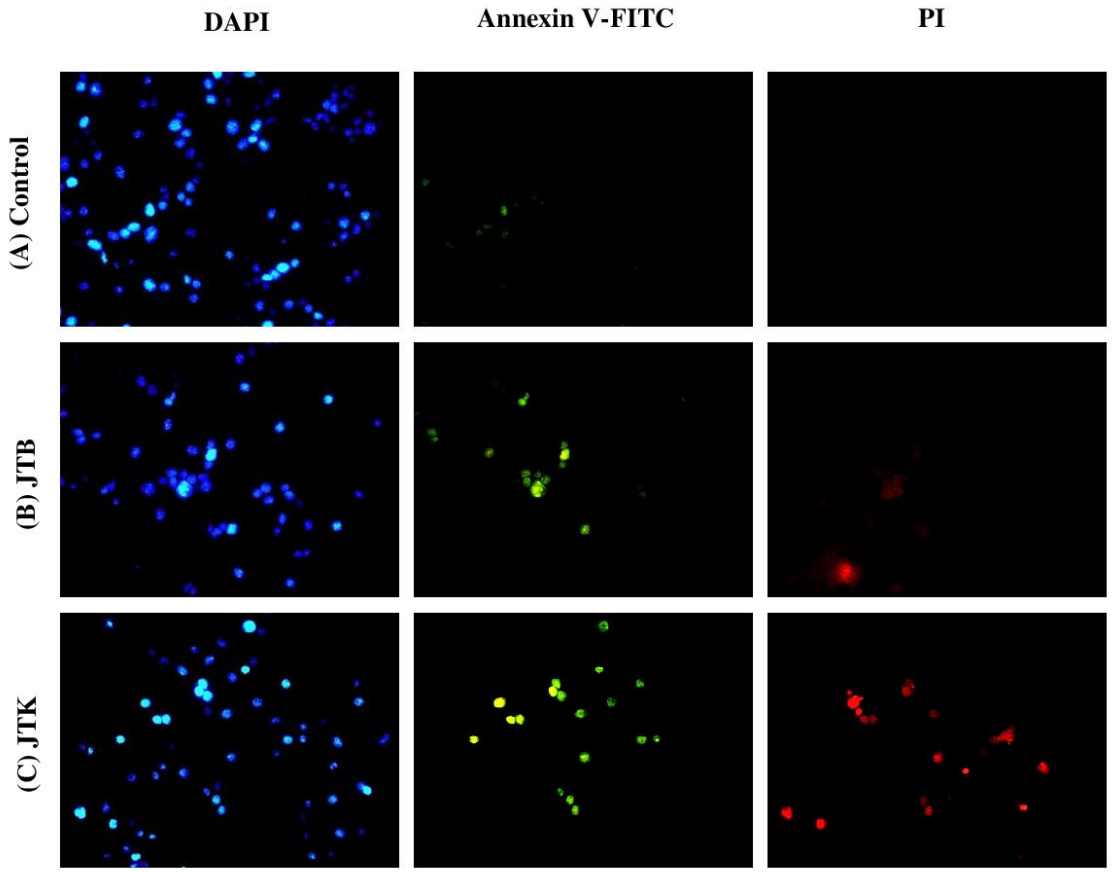

Figure 3 Apoptosis analysis of T47D cells by fluorescent staining. (A) Untreated control, (B) JTB-treated, and (C) JTK-treated cells (40× magnification). Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue), while apoptotic cells were detected with Annexin V-FITC (green). Propidium iodide (PI; red) distinguished necrotic/late apoptotic populations. JTK treatment demonstrated the strongest induction of both apoptosis and necrosis compared to JTB and control groups.

Apoptosis assay by cell staining

T47D cells apoptosis by cell staining results based on treatment JTB and JTK were determined and shown in **Figure 3. Figure 3** shows the staining results of T47D cells after treatment**.** Visualization on photomicrograph with blue colour shows cell nuclei stained with DAPI, green colour shows annexin V-FITC positive fluorescence and red colour shows PI positive fluorescence. Annexin V-FITC negative and PI negative indicate live cells, annexin V-FITC positive and PI negative indicate early apoptosis, annexin V-FITC positive and PI positive indicate late apoptosis, and annexin V-FITC negative and PI positive indicate cells undergo necrosis**.**

The untreated control **(Figure 3(A))** is T47D cells without treatment that show viable cells in large numbers and can proliferate, as evidenced by negative visualization on annexin V-FITC and PI**.** Furthermore, JTB treatment **(Figure 3(B))** show positive visualization on annexin V- FITC and PI, but the cells that emit colour tend to be few which means only a few of the cells experience apoptosis**.** JTK treatment **(Figure 3(C))** shows positive results of annexin V-FITC and PI with visualization of a larger number of cells**.** This is thought to indicate that JTK can induce higher apoptosis compared to JTD**.** Overall, JTK treatment induces the highest apoptosis, as evidenced by the results of photomicrograph visualization using annexin V-FITC and PI staining**.**

The ability to induce apoptosis in T47D cells is related to the kombucha fermentation process**.** Microbes produce enzymes that can hydrolyze polyphenolic compounds and produce organic acids **[**40**].** These active components have the ability as anti-cancer**.** JTK contains *D-saccharic acid1,4-lactone* **(**DSL**)** which is not found in unfermented beverages **[**36,41**].** DSL can inhibit the activity of the enzyme glucuronidase**.** The enzyme glucuronidase can hydrolyze glucuronides and produce cancer-causing aglycones**.** In addition, compounds of the phenolic group, flavonoids, tannins, alkaloids, and glycosides play a significant antitumor function **[**42**].** The compounds are produced during kombucha fermentation and selectively kill rapidly dividing cells, target abnormally expressed molecular factors, relieve oxidative stress, modulate cell growth factors, inhibit angiogenesis of cancer tissues, and induce apoptosis **[**43**].**

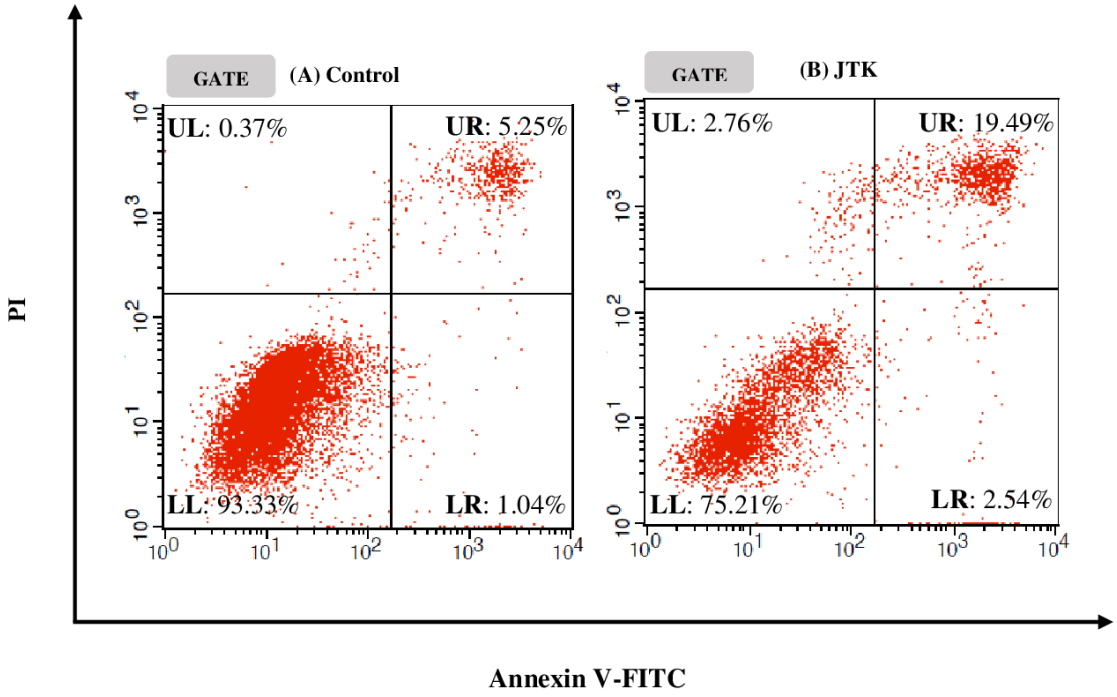

Figure 4 Flow cytometry analysis of T47D cells using Annexin V-FITC and Propidium Iodide (PI). (A) Control, (B) JTK-treated cells. The LL (Lower Left), LR (Lower Right), UR (Upper Right) and UL (Upper Left) quadrants indicate live cells, early apoptosis, late apoptosis and necrosis. JTK treatment has better anti-cancer ability compared to the control.

Apoptosis assay by flow cytometry

T47D cell apoptosis by *flow cytometry* analysis results based on treatment JTK was determined and shown in **Figure 4**. **Figure 4(A)** show the results of flow cytometry analysis of T47D cells in the control have the number of viable cells at 93.33 %; early apoptosis at 1.04 %; late apoptosis at 5.25 %; and necrosis at 0.37 %. Live cell data on the control showed high value compared to the treatment of JTK. Furthermore, JTK treatment in **Figure 4(B)** shows the number of viable cells at 75.21 %; early apoptosis at 2.54 %; late apoptosis at 19.49 %; and cell necrosis at 2.76 %. These results also show that JTK treatment has anti-cancer ability because it can kill cancer cells with more than 22 % cell apoptosis.

JTK showed the ability to trigger apoptosis in T47D cells (**Figure 4**). Treatment with JTK exhibited superior anti-cancer activity compared to the control, as evidenced by an apoptosis rate exceeding 22 %. JTK contained 11 bioactive compounds that are not found in JTB, consisting of curcuminoids and their derivatives including demethoxycurcumin (DMC), tetrahydrocurcumin (THC), and curcumin glucuronide; and flavonoids in the form of quercetin which are thought to have good anti-cancer activity [41]. These compounds are metabolites resulting from the metabolism of starter microorganisms. The general mechanism of phytochemical compounds from JTK fermentation that act as anti-cancer agents is to absorb reactive oxygen species (ROS) as well as increase the activity of antioxidant enzymes (e.g. catalase and superoxide dismutase enzymes) in cells. These compounds can block the metabolic conversion of pro-carcinogens and modulate cellular events and signaling involved in cancer cell growth, invasion, and metastasis [43]. Moreover, the expression of the tumor suppressor protein p53 was found to be highly regulated in response to DNA damage, facilitating DNA repair; if repair fails, p53 activation leads to apoptosis and subsequent cell death [44]. Anti-cancer compounds also downregulate inflammatory mediators such as NF-κB and IL-8 [45]. Furthermore, these agents suppress survivin and other pro-inflammatory gene expressions. Survivin is an intracellular protein encoded by the survivin gene that belongs to the apoptosis inhibitor family gene, and is highly expressed in the most common human tumor cells. Downregulation of survivin genes and dissociation of the cytoplasmic membrane induce apoptosis and cell death in treated cancer cells [46].

Conclusions

Kombucha derived from Javanese turmeric exhibits superior physicochemical properties compared to the unfermented beverage. Apoptosis analysis demonstrated that JTK induced apoptosis in over 22 % of the T47D cell population, indicating a promising cytotoxic potential. These findings indicate that fermentation enhances the physicochemical characteristics and anti-cancer properties of Javanese turmeric, suggesting that kombucha produced from this plant may serve as a promising complementary beverage for breast cancer therapy.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., Grammarly and ChatGPT by OpenAI) solely for language editing and grammar refinement during the preparation of this manuscript. No AI tools were used for content generation, data analysis, or interpretation. The authors take full responsibility for the integrity and conclusions of the work.

CRediT Author Statement

Elok Zubaidah: Conceptualization, methodology, Supervision, Validation, and Funding acquisition.

Yuliatin Hasfiani: Conceptualization, Data curation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

Hidayat Sujuti: Conceptualization, methodology, Supervision, and Validation.

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