Cytotoxic Effects of Cinnamon Powder and Lemongrass Oil-Enriched Roselle Tea on T47D Breast Cancer Cells

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Abstract

Breast cancer is the most prevalent cancer among women and is the leading cause of cancer-related mortality worldwide. This underlines the importance of preventive strategies to reduce the risk. Harnessing natural products like tea could be an alternative preventive strategy. In this study, the chemopreventive potential of roselle flower tea (*Hibiscus sabdariffa* L.) enriched with cinnamon oil (*Cinnamomum burmannii*) and lemongrass (*Cymbopogon citratus*) was evaluated. The tea was formulated using a stirred boiler tank and tested for its cytotoxicity against T47D cells using the MTT method. The cytotoxicity test showed that with the addition of cinnamon oil and lemongrass, roselle tea had more significant cytotoxic activity against T47D cancer cells than pristine roselle tea. Adding 5 g of cinnamon and lemongrass oil vapor for 15 seconds into the tea showed the highest cytotoxicity, with an IC_{50} of 730 µg/mL. This value is almost three times higher than without adding cinnamon oil and lemongrass. In conclusion, adding cinnamon oil and lemongrass to the roselle tea formulation can increase cytotoxic activity against T47D cancer cells, indicating its potential as a natural anti-cancer agent.

Keywords: *Chemopreventive, Cinnamon, Lemongrass, Roselle Tea, T47D Cells.*

INTRODUCTION

Cancer remains a primary cause of morbidity and mortality worldwide, constituting a formidable public health challenge. The prevalence of cancer has been exacerbated by rapid population growth and shifting lifestyles, positioning it as the second leading cause of death among non-communicable diseases globally (Ma, *et al*., 2021). According to the International Agency for Research on Cancer (IARC), a branch of the World Health Organization (WHO), breast cancer is among the most frequently diagnosed cancers, with an incidence rate of 11.7% (Sung, *et al*., 2021). The urgent demand for effective cancer treatment strategies has catalyzed a burgeoning interest in approaches based on natural products.

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Chemoprevention, which involves using natural or synthetic agents to prevent cancer development, aims to halt, delay, or reverse carcinogenesis, presenting a promising strategy to reduce cancer incidence (Shankar, *et al*., 2022). Natural products, particularly those derived from plants, have demonstrated significant potential in chemoprevention due to their bioactive compounds with anticarcinogenic properties (Haque, *et al*., 2021). Roselle tea, derived from the petals of the roselle flower (*Hibiscus sabdariffa* L.), is one such natural ingredient investigated for its chemopreventive properties. Rich in polyphenols, flavonoids, and anthocyanins, roselle tea is recognized for its antioxidant (Bevan, *et al*., 2023; Essiedu & Kovaleva, 2024), anti-inflammatory (Bayani, *et al*., 2018), and anticancer activities (Yasmin, *et al*., 2023).

Previous research has demonstrated the potential of roselle flowers (*Hibiscus sabdariffa*) in inhibiting the proliferation of various cancer cell lines, including breast cancer cells. Notably, a study by Kaulika & Febriansah (2019) identified anthocyanins in roselle flower petals as promising chemopreventive agents against T47D breast cancer cells. However, the chemopreventive efficacy of roselle tea against T47D breast cancer cells remains suboptimal, necessitating further enhancement. Incorporating bioactive compounds from additional natural sources is being explored to augment the chemopreventive activity of roselle tea.

Cinnamon (*Cinnamomum burmannii*) and lemongrass (*Cymbopogon citratus*) are renowned for their potent anticancer properties. Cinnamon, rich in cinnamaldehyde and polyphenolic compounds, has demonstrated cytotoxic effects against breast cancer cells through apoptosis induction, inhibition of cell proliferation, and modulation of cellular pathways (Larasati & Meiyanto, 2019; Anjarsari, *et al*., 2018; Sharma, *et al*., 2022).

Cinnamaldehyde from cinnamon has also demonstrated cytotoxicity against breast cancer cells by impeding cell proliferation, invasion, and migration, altering cytoplasmic morphology, and promoting apoptosis (Liu, *et al*., 2020). This effect is mediated through downregulation of the antiapoptotic protein c-MYC (Yi, *et al*., 2022), elevation of intracellular ROS via mitochondrial membrane disruption, and activation of caspase-8 (Rad, *et al*., 2015).

Similarly, citral, the main bioactive constituent in lemongrass oil, significantly inhibits the proliferation of MCF-7 breast cancer cells by inducing apoptosis and reducing the ALDH-positive cell population. This action involves downregulation of β-catenin and cyclin D1 proteins, concomitant upregulation of phospho-β-catenin, and modulation of the Wnt/β-catenin pathway (Rusli, *et al*., 2022; Thomas, *et al*., 2016). Despite their individual chemopreventive potential, the synergistic effects of combining roselle tea, cinnamon, and lemongrass oil remain unexplored.

In this study, the approach used to combine cinnamon powder and lemongrass oil with roselle tea involved mixing two components, solid and gas using a stirred boiler tank, which was previously developed by Cahyono, *et al*., (2021). This method was chosen to prevent significant changes in the appearance and taste of the tea, which could occur if the oil were added directly in excessive amounts. Building on this, the study aims to evaluate the enhanced efficacy of roselle tea combined with cinnamon and lemongrass oils in inhibiting the growth of T47D breast cancer cells compared to roselle tea alone. By integrating multiple bioactive components, the findings are anticipated to provide valuable insights into the development of more effective natural-based chemopreventive agents for breast cancer.

METHODS

Ethical Concern

All *in vitro* cytotoxic tests in this study were conducted following ethical guidelines and approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Negeri Semarang, with reference number 055/KEPK/FK/KLE/2024.

Materials

Roselle plants, sourced from the Roselle Tea & Coffee Garden in Central Java, Indonesia, and cinnamon from Boyolali, Indonesia, have been authenticated by biologists at the Plant Taxonomy Laboratory of Universitas Negeri Semarang, with the entry number 232/UN.37/SHP/Lab. Taksonomi Tumbuhan/XI/2023. Lemongrass oil was procured from the distillation processes conducted at Rumah Atsiri Indonesia (RAI), Tawangmangu, Indonesia. T47D cells were acquired from the Cancer Chemopreventive Research Center (CCRC), Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

Identification of Chemical Compounds in Tea Raw Materials

The phytochemical profile of roselle flowers was evaluated by screening the presence of alkaloids, flavonoids, saponins, tannins, and triterpenoids. The extraction of roselle flowers for the phytochemical screening was performed using the infusion method. Specifically, 40 grams of roselle flowers were immersed in 400 mL of distilled water, followed by heating the mixture on a hotplate at 100°C for 20 minutes. After cooling, the mixture was filtered using filter paper (Nguyen & Chuyen, 2020) and the filtrate was evaporated to yield a concentrated extract.

Alkaloid compounds were identified using Mayer's and Dragendorff's reagents. The extract was dissolved in hot distilled water and placed into two test tubes. Each tube was then treated with 3 drops of 2N HCl and heated in a water bath for 2 minutes. After heating, the first tube was reacted with 3 drops of Dragendorff's reagent, while the second tube was reacted with 3 drops of Mayer's reagent. The formation of a white precipitate indicated a positive result for Mayer's test, whereas a positive result for Dragendorff's test was marked by an orange precipitate (Sari & Aryantini, 2018).

The identification of flavonoids was performed by diluting 1 mL of extract in hot distilled water, which was then boiled in a water bath for 5 minutes. After boiling, 3 drops of 10% HCl and 0.02 mg of magnesium powder were added. A positive result for flavonoids was indicated by a color change in the solution to red, orange, or yellow (Sari & Aryantini, 2018).

The identification of saponins was carried out by adding 1 mL of extract to hot water, followed by vigorous shaking. Subsequently, two drops of concentrated HCl were added. A positive result for saponins was confirmed by forming a stable foam for approximately 5 minutes.

The identification of tannins was conducted by dissolving 1 mL of extract in hot distilled water and placing it in a test tube. Two drops of $FeCl_3$ were then added. A positive result was indicated by a color change to dark green or blackish-green. The identification of triterpenoids was performed by adding 3 drops of Liebermann-Burchard reagent to the extract solution. A positive result was indicated by the appearance of red or purple colouration in the sample solution (Sari & Aryantini, 2018).

The chemical constituents of cinnamon and lemongrass oil were elucidated using untargeted GC-MS (Shimadzu GC-2010 Plus).

Tea Formulations

Following initial testing, the ingredients underwent preparation for formulation. Roselle flowers were meticulously washed and their petals carefully selected before drying in an oven at 40°C for 6 h. Concurrently, cinnamon bark underwent drying and subsequent grinding to yield cinnamon powder. The ingredients were then processed in a stirred broiler tank. The evaporation tube containing lemongrass oil was heated to a temperature of 70°C, maintaining a steam pressure of 2 bar. The automatic stirring tube, containing 125 grams of roselle tea and cinnamon powder, was subjected to continuous stirring at a fixed speed of 20 rpm for 15 minutes, followed by lemongrass oil vapor exposure from the evaporation tube. Variations of the formula are detailed in Table 1.

Table 1. Variation of Roselle Herbal Tea Formulation.

Formulation	The exposure time of lemongrass oil vapor (second)	Weight of cinnamon powder (g)
F1	0	0
F2	0	15
F3	5	10
F4	5	15
F5	10	10
F6	15	5

Cell Line Culture

T47D cells were cultured at the Parasitology Laboratory, FK-KMK, Universitas Gadjah Mada, Yogyakarta, Indonesia. The cells were maintained in Roswell Park Memorial Institute (RPMI) medium (Gibco, USA), supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) penicillin-streptomycin (Gibco). The cultures were incubated at 37°C in a 5% CO_2 atmosphere.

Cytotoxic Assay

The cytotoxicity of roselle tea against T47D cancer cells was evaluated using the MTT assay, following established protocols CCRC (2009, 2012). T47D cells were cultured to 80% confluence in a CO_2 incubator, then detached using trypsin-EDTA (0.25%) and resuspended in RPMI media. After cell counting using a hemacytometer, cells were seeded at 1×10^4 cells per well in a 96-well microplate, except for the media control well, and incubated for 24 h. A test sample (20 mg) was dissolved in 200 µL DMSO and added to the wells in concentration series (1000, 500, 250, 125, 62.5 µg/mL) in triplicate, with roselle flowers as the negative control. While cells without any treatment were used as the untreated control. After 24 h of incubation, cell viability was assessed using the MTT assay. Briefly, MTT reagent (100 µL) was added to each well and incubated for 4 h, followed by 10% SDS in 0.01 N HCl (100 µL) to solubilize formazan crystals. The plate was then wrapped in foil and incubated overnight at room temperature in the dark. Absorbance readings were performed at 559 nm using an ELISA reader to quantify cell viability. This experimental approach adheres to standardized methods for assessing the cytotoxic effects of natural compounds on cancer cell lines and ensures reliable data for further analysis and interpretation. The percentage of viable cells is calculated using Equation 1.

$$\%\ \text{viable cells} = \frac{(\text{Abs treatment} - \text{Abs media control})}{(\text{Abs cell control} - \text{Abs media control})} \times 100\%$$

The percentage of viable cells was then analyzed to determine the IC_{50} value.

The tea sample with the highest cytotoxic activity was then further analyzed for its chemical compounds. The tea was extracted with boiling water. Following extraction, the tea was filtered and transferred to a separatory funnel. Subsequently, n-hexane was added and vigorously shaken until phase separation occurred. The aqueous phase was decanted, transferred to a vial, and subjected to solvent evaporation at ambient temperature (Noraida, *et al*., 2021). The resulting dried extract was subsequently analyzed using a GC-MS (Shimadzu GC-2010 Plus).

Moisture Content Analysis

The moisture content analysis of roselle tea was conducted following the standards specified in SNI 3836:2013, utilizing the oven drying method. Porcelain cups, labelled with specific sample codes, were heated in a Memmert TV30U oven at a controlled temperature of (105±2)°C for one hour to achieve thermal equilibrium. Each cup was then transferred to a desiccator and weighed to determine its initial weight (W0). A precisely measured 2 g sample was placed in the cup, and the

combined weight was recorded (W1). The loaded cup was returned to the Memmert TV30U oven and further heated at (105±2)°C for three hours. After each heating cycle, the cup was transferred to a desiccator for approximately 30 minutes to cool to ambient temperature before being reweighed until a constant weight (W2) was obtained, with a precision of ≤1 mg. This procedure was repeated three times to ensure the reproducibility and accuracy of the results (SNI, 2013). The moisture content (%) was calculated using Equation 2.

$$\text{Moisture Content} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\%$$

W0 = weight of the empty crucible (g)
W1 = weight of the crucible+sample before drying (g)
W2 = weight of crucible + sample after drying (g)

Total Ash Content Analysis

The ash content analysis was conducted according to SNI 3836:2013 using the thermogravimetric method. Initially, the porcelain ash cup was heated in a Memmert TV30U oven at (105±2)°C for approximately one hour, followed by equilibration in a desiccator until reaching ambient temperature. The initial weight of the empty porcelain cup (W0) was recorded, and subsequently, 2 g of the sample was placed in the cup and weighed (W1). The cup containing the sample was then heated in a Nabertherm N3/R furnace at (525±25)°C until complete ashing occurred, indicated by the formation of white ash. Post-ashing, the cup was allowed to cool in a desiccator to room temperature and reweighed (W2). This procedure was repeated three times following the specifications outlined in SNI 3836:2013. This procedure was repeated three times in accordance with the specifications outlined in SNI 3836:2013. The ash content was determined using Equation 3.

$$\text{Ash Content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

W0 = weight of the empty crucible (g)
W1 = weight of crucible + sample before ashing (g)
W2 = weight of crucible + sample after ashing (g)

RESULTS

Determination of Chemical Compounds in Tea Raw Materials

Phytochemical analysis of roselle flower water extract indicated the presence of flavonoids, tannins, and triterpenoids, while alkaloids and saponins were absent. These findings corroborate previous reports documenting the phytochemical profile of roselle extracts, which similarly identified flavonoids, tannins, and triterpenoids (Sari & Aryantini, 2018). The result of the phytochemical analysis of roselle flower extract are presented in Table 2.

In the meanwhile, the analysis of volatile components in lemongrass essential oil using GC-MS revealed fourteen distinct compounds, with (E)-Citral constituting the majority at 50.15% and (Z)-Citral at 33.58% (Figure 1). Conversely, cinnamon powder predominantly contains cinnamaldehyde.

Table 2. Phytochemical analysis of roselle extract.

Secondary Metabolites	Reagent	Observation	Result
Alkaloids	Dragendorff	Red solution	-
	Mayer	Red solution	-
Flavonoids	HCl + Mg	Orange solution	+
Tannins	$FeCl_3$	Dark-green solution	+
Triterpenoids	Acetic anhydride + H_2SO_4	Dark-red solution	+
Saponins	Hot water + HCl	Unstable foam	-

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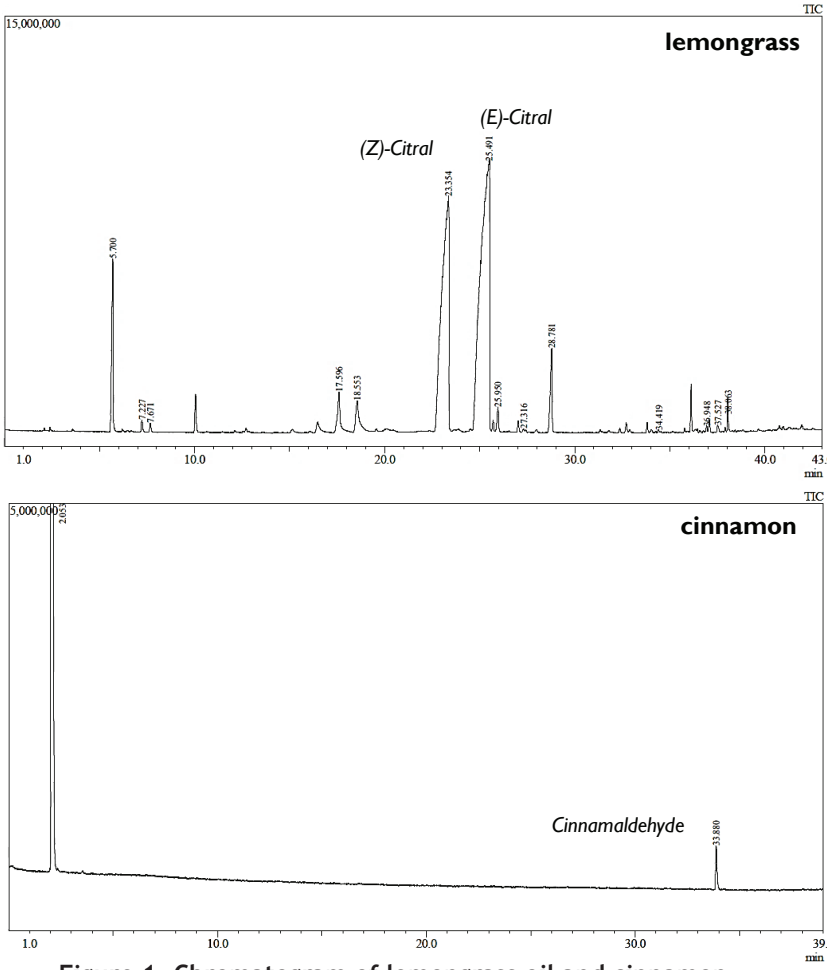

Figure 1. Chromatogram of lemongrass oil and cinnamon.

Tea Formulations

Combining roselle tea and cinnamon powder, followed by the addition of lemongrass oil, induces a notable darkening in the color of the roselle tea (Figure 2) and imparts a distinct lemongrass aroma, indicative of thorough blending of the ingredients. The incorporation of cinnamon powder, known for its natural pigments such as coumarin and tannin, significantly influences the color transformation of roselle tea owing to the chemical characteristics inherent to cinnamon. Specifically, coumarin, a constituent of cinnamon, imparts a brown hue to the beverage, while tannin facilitates color modification through oxidative reactions with compounds in roselle tea (Arismawanti, *et al*., 2021). Additionally, lemongrass oil, containing citral compounds, intensifies the citrus aroma, enhancing the overall sensory experience.

Figure 2. Physical appearance of tea powder. Left (F1, only roselle) and right (F6, roselle + lemongrass vapor (15s exposure) + 5 g cinnamon powder).

Cytotoxic Activity of Roselle Herbal Tea against T47D Cells

The cytotoxic activity of roselle tea and cinnamon and lemongrass-enriched roselle tea against T47D cells was analyzed using the MTT method (Figure 3).

The highest cytotoxic activity in roselle tea with the addition of cinnamon and lemongrass oil vapor was observed in the F6 formulation, showing a moderate cytotoxic effect with an IC_{50} value of 730 µg/mL (Table 3). F5 formulation also showed a moderate cytotoxic effect but with a higher IC_{50} value (985.2 µg/mL). On the other hand, the negative control (F1), which is pristine roselle powder showed a weak cytotoxic effect with an IC_{50} value of 2066.6 µg/mL.

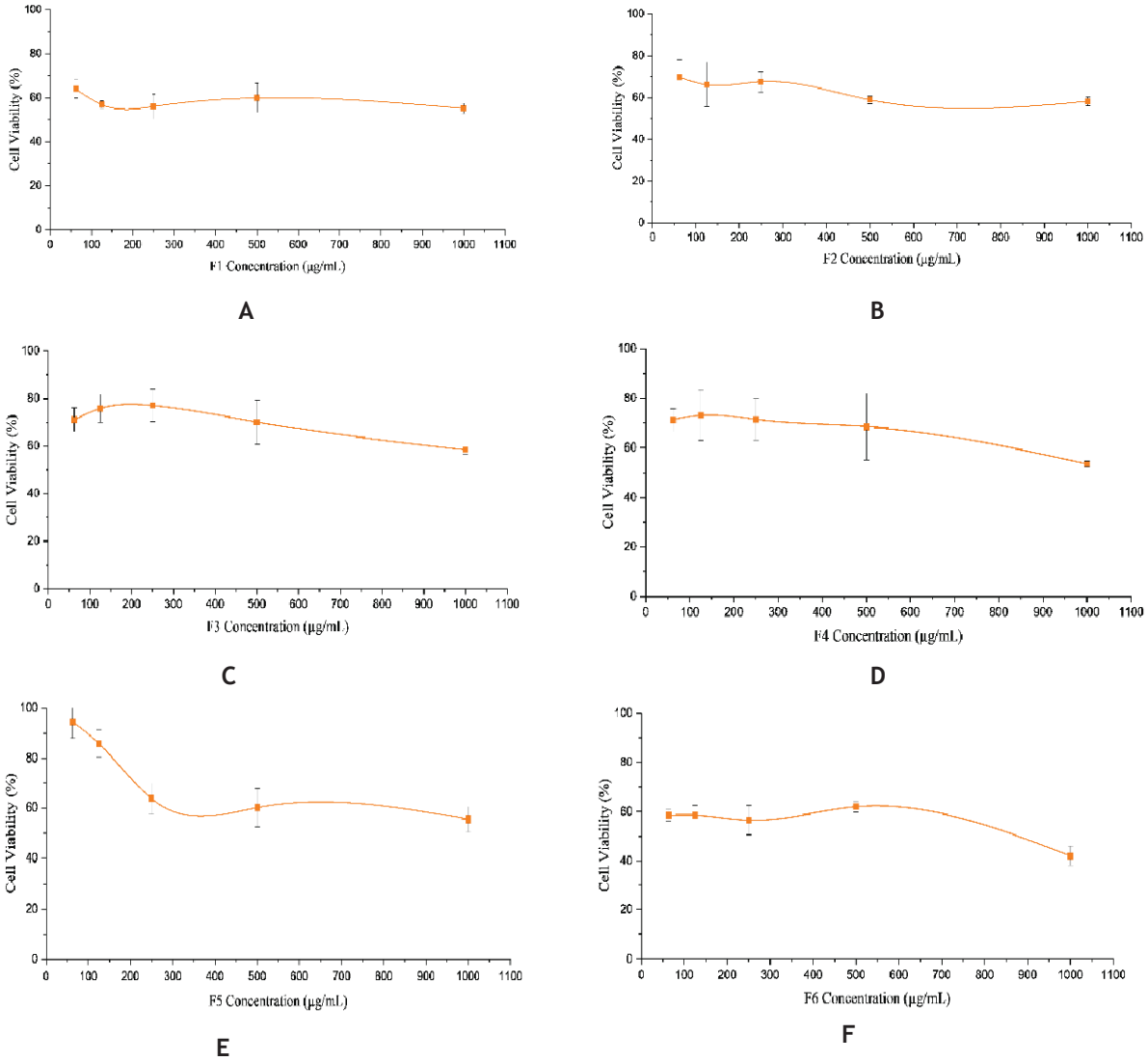

Figure 3. Cytotoxicity profile of roselle tea F1 (A), F2 (B), F3 (C), F4 (D), F5 (E), F6 (F). 5x10^4 T47D cells were seeded in each well of microplate 96-wells and treated with a concentration of tea series of 62.5-1000 µg/mL.

Table 3. IC_{50} value of the tea.

Formulation	Additional Materials		IC_{50} value (µg/mL)
	Cinnamon (g)	Lemongrass oil (exposure time, s)	
F1	0	0	2,066.6
F2	15	0	1,554.6
F3	10	5	1,593.8
F4	15	5	1,260.0
F5	10	10	985.2
F6	5	15	730.0

Moisture Content and Ash Total Content of Roselle Tea

The moisture and total ash content tests were carried out on roselle tea F6, which had the highest cytotoxic activity. The results of the water content and total ash content of roselle tea F6 formulation are presented in Table 4.

Based on the analysis of the moisture content, it is known that the tea has a water content of 13.628% and a total ash content of 8.66%. This amount is much higher than the maximum water content determined by SNI 3836:2013 for dry-packaged tea, which is 7-8% (Table 4).

Table 4. Water content and total ash content of roselle tea F6.

	Result (%)	SNI (%)
Water Content	13.628	7.00-8.00
Total Ash Content	8.66	8.00

Chemical Analysis of Roselle Tea F6

GC-MS analysis was performed on the optimized roselle tea formulations (F6) to evaluate the presence of citral and cinnamaldehyde. The chromatogram (Figure 4) revealed substantial absorption of lemongrass, evidenced by a prominent peak of citral. Conversely, cinnamaldehyde exhibited a comparatively minor peak. Specifically, the presence of *(Z)-citral* and *(E)-citral* was quantified at 31.56% and 58.90%, respectively, whereas cinnamaldehyde constituted approximately 4% of the compound composition (Table 5). Additionally, nerol was detected at 6.35%, which is likely derived from lemongrass oil, as nerol is a monoterpenoid commonly found in lemongrass oil.

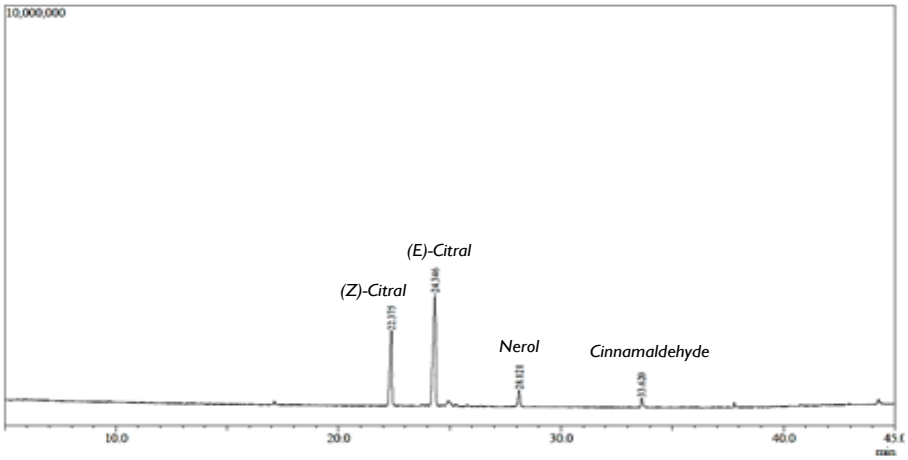

Figure 4. Chromatogram of roselle tea F6.

Table 5. Peak report of roselle tea F6 compound.

Peak	Retention time (second)	% Area	Compound
1	22.375	31.56	*(Z)-citral*
2	24.346	58.90	*(E)-citral*
3	28.121	6.35	*Nerol*
4	33.620	3.20	*Cinnamaldehide*

DISCUSSION

Chemopreventive agents are substances applied to lower the risk of developing cancer. They can be drugs, vitamins, or supplements that are used to prevent the rise of cancer in healthy people or people who are at risk due to a genetic history of cancer. Chemopreventive agents that taste good are more acceptable for patients. Tea is part of people's culture and can be used as a means to provide chemopreventive substances, including roselle flower tea.

Roselle flower tea, rich in anthocyanin, is recognized for its potential chemopreventive properties. To enhance these effects, additional natural ingredients such as cinnamon and lemongrass oil vapor are incorporated. These additional ingredients contain aldehyde groups, which are well-documented for their cytotoxic effects, inducing apoptosis, necrosis, cell cycle arrest, cellular dysfunction, and reduction in tumor size (Elshafie & Camele, 2017; Sharma, *et al*., 2022). While citral, as the main bioactive of lemongrass oil, induces oxidative stress and increases reactive oxygen species (ROS). Oxidative stress also activated the ERK1/2-AKT-PI3K-NFκB signaling pathways leading to cell death and apoptosis. Inhibition of the enzyme ALDH1A3 is involved in converting retinal to retinoic acid (RA) and generates DNA damage in cancer cells (Bailly, 2020).

The chemopreventive efficacy of roselle tea supplemented with cinnamon and lemongrass vapor has been investigated against T47D breast cancer cells using the MTT assay to assess cytotoxic activity across varying ingredient concentrations. The experiment employed pristine roselle tea as a negative control (F1). Results indicated that F1 exhibited limited cytotoxicity with an IC_{50} value of 2,066.6 µg/mL. Conversely, the incorporation of cinnamon and lemongrass vapor notably enhanced the cytotoxic potency of the herbal infusion, particularly evident in F5 and F6 formulations, which demonstrated IC_{50} values of 985.2 µg/mL and 730 µg/mL, respectively. This finding indicates that formulas F6 and F5 are included in the moderate cytotoxic group (Prayong, *et al*., 2008). It means that the formulas have the potential as a chemopreventive agent but not as an anticancer. The National Cancer Institute (NCI) classifies a compound as anticancer if it has an IC_{50} of less than 20 µg/ml.

Increasing the concentration of bioactive compounds is a key strategy to enhance the cytotoxic activity of the roselle tea formulation. Optimizing the cinnamon and lemongrass oil ratio in the formulation could enhance their synergistic effects, particularly by increasing citral levels, which are known to exhibit strong cytotoxicity against breast cancer cells.

These outcomes underscore the promise of roselle tea combined with cinnamon and lemongrass vapor as a potential chemopreventive agent for breast cancer. This aligns with previous studies by Patel, *et al*. (2015) that highlighted synergistic benefits from natural compound combinations in enhancing cytotoxic efficacy against breast cancer cells. The enhanced cytotoxic activity observed in formulations F5 and F6 suggest possible interactions between the bioactive compounds, but further validation is required to definitely determine whether the interaction is additive or

synergistic, such as using Combination Index (CI) or isobologram analysis.

In addition to its cytotoxic properties, the moisture and ash content of the tea formulation is an important factor influencing its quality and shelf life. The analysis revealed that the tea had a moisture content of 13.628% and a total ash content of 8.66%. This moisture level exceeds the maximum water content set by SNI 3836:2013 for dry-packaged tea, which is 7–8%. A higher moisture content can increase the risk of microbial growth, degradation of bioactive compounds, and reduced shelf stability. Further optimization of the drying process is necessary to reduce the moisture content within the acceptable standard range, ensuring product stability while maintaining its bioactive properties, such as extending the duration of the drying process that could effectively reduce the moisture content to meet the standard range. The elevated ash content suggests a significant presence of mineral components, which may contribute to the overall nutritional profile of the tea.

CONCLUSION

Based on the research, it is evident that roselle tea supplemented with cinnamon and lemongrass oil exhibits higher cytotoxic activity compared to pristine roselle tea. Among the assessed tea formulas, the F6, involving 5 g of cinnamon and a 15-second exposure of lemongrass oil vapor, resulting the highest cytotoxic activity, characterized by an IC_{50} value of 730 µg/mL, indicating moderate cytotoxicity. This finding suggest the potential of treated roselle tea as a chemopreventive agent against breast cancer.

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REFERENCES

Anjarsari, E.Y., Kristina, N., Larasati, Y.A., Putri, D.D.P., and Meiyanto, E., 2013, Synergistic effect of cinnamon essential oil (*Cinnamomum burmannii)* and doxorubicin on T47D cells correlated with apoptosis induction, *Indonesian Journal of Cancer Chemoprevention,* **4**(1), 450-456.

Arismawanti, P., Irmayanti, and AR, C., 2021, Formulation For Making Edible Spoon With Additional Variants of Natural Dye Extract and Cinnamon Powder (*Cinamomum Burmanii*) as Anti-Microbial, *Serambi Journal of Agricultural Technology*, **3**(2), 96–105.

Bailly, C., 2020, Targets and pathways involved in the antitumor activity of citral and its stereo-isomers, *European Journal of Pharmacology*, **871**.

Bayani, G.F.,E, Marpaung, N.L.E., Simorangkir, D.A.S., Sianipar, I.R., Ibrahim, N., Kartinah, N.T., *et al.*, 2018, Anti-inflammatory Effects of *Hibiscus Sabdariffa Linn*. on the IL-1ß/IL-1ra Ratio in Plasma and Hippocampus of Overtrained Rats and Correlation with Spatial Memory, *The Kobe Journal of Medical Sciences*, **64**(2), 73–83.

Bevan, P., Pastor, M.V., Almajano, M.P., and Codina-Torrella, I., 2023, Antioxidant and Antiradical Activities of *Hibiscus sabdariffa* L. Extracts Encapsulated in Calcium Alginate Spheres, *Polymers*, **15**(7), 1–19.

Cahyono, E., Kurniawan, C., and Anggraito, U., 2021, Vapor-solid Mixer Design for Cinnamal Tea Production, *Journal of Physics: Conference Series*, **1918**(3), 1–6.

CCRC, 2009a, Prosedur Tetap: Perhitungan Sel, Cancer Chemoprevention Research Center Farmasi UGM Yogyakarta, 1–4.

CCRC, 2009b, Prosedur Tetap Panen Sel, Cancer Chemoprevention Research Center, Fakultas Farmasi UGM, 1–3.

CCRC, 2009c, Prosedur Tetap Preparasi Sampel, Fakultas Farmasi UGM, 1–3.

CCRC, 2012, Uji Sitotoksik Metode MTT, Cancer Chemoprevention Research Center Fakultas Farmasi UGM, 6–9.

Elshafie, H.S., and Camele, I., 2017, An Overview of The Biological Effects of Some Mediterranean Essential Oils on Human Health, *BioMed Research International*, **1**(1), 1–14.

Essiedu, J.A., and Kovaleva, E.G., 2024, Physicochemical, antioxidant activity, and sensory characteristics of mead produced with Hibiscus sabdariffa and Betula pendula (Birch sap), *Biocatalysis and Agricultural Biotechnology*, **58**, 103189.

Haque, A., Brazeau, D., and Amin, A.R., 2021, Perspectives on natural compounds in chemoprevention and treatment of cancer: an update with new promising compounds, *European Journal of Cancer*, **149**(1), 165–183.

Kaulika, N., and Febriansah, R., 2019, Chemopreventive Activity of Roselle's Hexane Fraction against Breast Cancer by *In-Vitro* and *In-Silico* Study, *Advances in Health Sciences Research*, **15**(1), 66–71.

Larasati, Y.A., and Meiyanto, E., 2018, Revealing the potency of cinnamon as an anti-cancer and chemopreventive agent, *Indonesian Journal of Cancer Chemoprevention*, **9**(1), 47-62.

Liu, Y., An, T., Wan, D., Yu, B., Fan, Y., and Pei, X., 2020, Targets and Mechanism Used by Cinnamaldehyde, the Main Active Ingredient in Cinnamon, in the Treatment of Breast Cancer, *Frontiers in Pharmacology*, **11**, 582719.

Ma, L., Zhang, M., Zhao, R., Wang, D., Ma, Y., and Ai, L., 2021, Plant Natural Products : Promising Resources for, *Molecules*, **26**(4), 933.

Nguyen, Q.V., and Chuyen, H.V., 2020, Processing of herbal tea from roselle (*Hibiscus sabdariffa* L.): Effects of drying temperature and brewing conditions on total soluble solid, phenolic content, antioxidant capacity and sensory quality, *Beverages*, **6**(1), 1–11.

Noraida, L., Bintang, M., and Priosoeryanto, B.P., 2021, N-hexane Extract and Fraction of Green Tea as Antiproliferation of MCM-B2 Breast Cancer Cells In Vitro, *Current Biochemistry*, **6**(2), 92–105.

Patel, P.B., Thakkar, V.R., and Patel, J.S., 2015, Cellular effect of curcumin and citral combination on breast cancer cells: Induction of apoptosis and cell cycle arrest, *Journal of Breast Cancer*, **18**(3), 225–234.

Prayong, P., Barusrux, S., and Weerapreeyakul, N., 2008, Cytotoxic activity screening of some indigenous Thai plants, *Fitoterapia*, **79**(7), 598-601.

Rad, S.K., Kanthimathi, M.S., Malek, N.A., Lee, G.S., Looi, C.Y., and Wong, W.F., 2015, Cinnamomum cassia Suppresses Caspase-9 through Stimulation of AKT1 in MCF-7 Cells but Not in MDA-MB-231 Cells, *PLoS One*, **10**(12), 1–22.

Rusli, M.E.F., Rosli, R., Ramli, U.B., Suhaimi, S.A., and Saad, N., 2022, The Potentiality of Citral in Targeting Breast Cancer Multicellular Tumour Spheroids (MTS), *Malaysian Journal of Medicine and Health Sciences*, **18**(2), 2636–9346.

Sari, F., and Aryantini, D., 2018, Karakter Spesifik dan Pengaruh Pemberian Oral Ekstrak Terpurifikasi Kelopak Rosella (*Hibiscus sabdariffa* L.) terhadap Makroskopis Organ Hepar Tikus Wistar, *Jurnal Wiyata*, **5**(1), 1–9.

Shankar, M., Swetha, M., Keerthana, C.K., Rayginia, T.P., and Anto, R.J., 2022, Cancer Chemoprevention: A Strategic Approach Using Phytochemicals, *Frontiers in Pharmacology*, **12**, 1–25.

Sharma, M., Grewal, K., Jandrotia, R., Batish, D.R., Singh, H.P., and Kohli, R.K., 2022, Essential oils as anticancer agents: Potential role in malignancies, drug delivery mechanisms, and immune system enhancement, *Biomedicine and Pharmacotherapy*, **146**(1), 1–36.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide

for 36 Cancers in 185 Countries, *CA: A Cancer Journal for Clinicians*, **71**(3), 209–249.

Thomas, M.L., de Antueno, R., Coyle, K.M., Sultan, M., Cruickshank, B.M., Giacomantonio, M.A., *et al.*, 2016, Citral reduces breast tumor growth by inhibiting the cancer stem cell marker ALDH1A3, *Molecular Oncology*, **10**(9), 1485–1496.

Yasmin, R., Gogoi, S., Bora, J., Chakraborty, A., Dey, S., Ghaziri, G., *et al.*, 2023, Novel Insight into the Cellular and Molecular Signalling Pathways on Cancer Preventing Effects of *Hibiscus sabdariffa* : A Review, *Journal of Cancer Prevention*, **28**(3), 77–92.

Yi, X., Pei, T., Li, S., and Huang, L., 2022, Cinnamic aldehyde induces apoptosis of breast cancer cells via STAT3/cMyc pathway, *Research Square*, **1**(1), 1–18.