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Effect of Acid-Base Composition on Physical Characteristics and Antioxidant Activity of "Beras Kencur" Effervescent Tablet

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Abstract

This study aimed to evaluate the effect of acid-base ratio on the physical characteristics and antioxidant activity of the beras kencur effervescent tablet formulation. The formulation determination was based on the percentage inhibition value, where the highest value (76.2%) was found in beras kencur at 10 mg/mL, so that 1.5 g is needed to achieve the highest potential of radical scavenging activity. In this case, 1.5 g of sample was then incorporated into three effervescent tablets. The acid-to-base ratios used in this study were 1: 1.25 (F1), 1: 1.50 (F2), and 1: 2.50 (F3). The tablets were evaluated for pH, dissolution time, total phenolic content (TPC), and antioxidant activity. The results showed that a higher proportion of base (F3) improved solubility. Meanwhile, antioxidant activity evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, and total phenolic content determined using Folin-Ciocalteau, showed that tablets with a higher proportion of acid exhibited higher total phenolic content. However, considering that all three samples have very strong antioxidant activity, it can be concluded that F3 is the optimal formulation.

Keywords : antioxidant, cancer, effervescent, kaempferia galanga, rice

INTRODUCTION

Non-communicable diseases (NCDs), including heart disease, cancer, diabetes, and chronic respiratory diseases, are the leading causes of global mortality, accounting for approximately 74% of deaths worldwide (Moremane et al., 2023). In Indonesia, the prevalence of NCDs has been increasing each year (Azim et al., 2024). The rise in cases includes cancer (1.8%), stroke (10.9%), chronic kidney disease (3.8%), diabetes mellitus (8.5%), and hypertension (34.1%). The primary factor contributing to this increase is an unhealthy lifestyle, which induces oxidative stress caused by excessive reactive oxygen species (ROS) (Astuti et al., 2023). However, the use of specific medical treatments for NDCs has caused different paradoxes and negative effects on the human body (Moremane et al., 2023). Therefore, the number of individuals who opt for natural products for control and prevention of NCDs is gradually rising as they have higher efficacy and fewer side effects compared to synthetic ones (Chigurupati et al., 2022).

Kaempferia galanga L., commonly known as "kencur" in Indonesia, is an aromatic medicinal plant native to India and widely distributed across Asia. Several studies have demonstrated that *Kaempferia galanga* rhizome extract possesses antioxidant, antibacterial, antitumor, and anti-inflammatory properties due to its rich phytochemical content, including phenolics, flavonoids,

terpenoids, diarylheptanoids, and dipeptides (Astuti et al., 2023). As antioxidants, the phenolic compounds mitigate free radicals by donating hydrogen from their hydroxyl group to peroxyl radicals (ROO*), forming a stable H-O bond with a single electron, as shown in Figure 1.

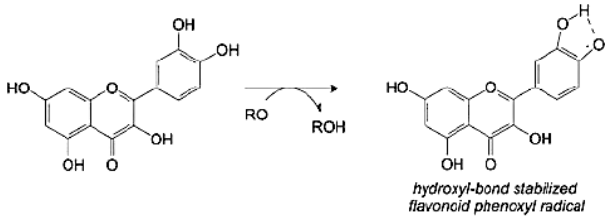

Figure 1. Radical scavenging mechanism

In recent years, consumption of *Kaempferia galanga* rhizome extract as a herbal drink has gained great attention among the Indonesian population. One of the Kaempferia galanga-based drinks originating from Java, Indonesia is "beras kencur". made from a combination of rice (beras) and Kae*mpferia galanga* (kencur) extract. It is part of the broader category of jamu, a traditional herbal medicine commonly consumed in Indonesia for its health benefits. "Beras kencur" is believed to aid digestion, boost energy, improve immunity, and relieve fatigue, while also having antioxidant and anti-inflammatory properties due to its phytochemical-rich ingredients. The drink, which has a slightly sweet and spicy flavor with a creamy texture from the rice, is commonly served warm to enhance its soothing and restorative effects. However, the serving temperature of "beras kencur" can damage and degrade the phenolic content. Therefore, formulating "beras kencur" into an effervescent tablet is an effective way to maintain antioxidant stability, as the effervescent form has a lower pH and can dissolve in room temperature water in less than 10 minutes. The research conducted by (Astuti et al., 2023) demonstrated that the effervescent formulation significantly affects the characteristics and antioxidant activities of effervescent tablet. However, the results remain too broad and not specific considering that the acid-to-base ratio is believed to have a significant influence to the antioxidant stability. Therefore, this study aims to investigate the impact of acid-base ratio on the physical stability and antioxidant activity of beras kencur effervescent tablets.

METHODS

Raw materials and reagents

Beras kencur powder was obtained from CV Suryo Gedhe Group (Kendal, Indonesia). It contains of *Kaempferia galanga*, rice, ginger, palm sugar, and white sugar (Figure 2 (a)). Additional materials including sodium bicarbonate ($NaHCO_3$), citric acid, tartaric acid, polyvinyl pyrrolidone (PVP), and maltodextrin were obtained from a local supplier (Nitra Kimia, Bantul, Indonesia), while chemicals used to analysis including 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), methanol, ethanol, sodium carbonate (Na_2CO_3), and folin-ciocalteu were purchased from Merck (Germany).

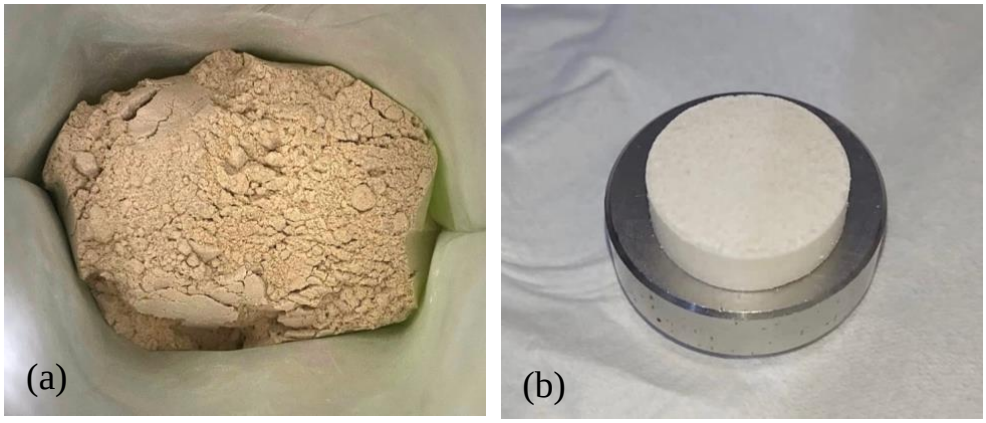

Figure 2. (a) *Beras kencur* powder from CV Suryo Gedhe Group, (b) Effervescent tablet

Formulation of effervescent tablets

In this study, three effervescent tablets were prepared with different ratios of acid and base (Table 1) to achieve the highest and most stable antioxidant activity while determining the ideal ratio for the fastest dissolution time. Each ingredient was weighed according to its proportions and ground using a mortar until the mixture became homogeneous. The addition and mixing of ingredients were carefully executed, starting with the base mixture (*beras kencur* powder, PVP, sodium bicarbonate, and maltodextrin) followed by the acid mixture (citric acid and tartaric acid). The homogeneous mixture of acids and bases was then dried in an oven (Memmert UN55, Germany) at 55 °C for 15 minutes. Finally, the dried mixture was moulded to effervescent tablets (Figure 2(b)). Completely, formulation of *beras kencur* effervescent tablet is presented in Table 2.

Table 1. Ratio of acid to base in three formulation *of beras kencur* effervescent

Formulation	Ratio of acid to base
F1	1 : 1.25
F2	1 : 1.50
F3	1 : 2.50

Table 2. Three formulation *of beras kencur* effervescent

	F1 (%)	F2 (%)	F3 (%)
Beras kencur powder	25.0	25.0	25.0
Citric acid	8.0	6.3	5.0
Tartaric acid	12.0	9.3	5.0
Sodium bicarbonate	25.0	23.4	25.0
Maltodextrin	25.0	34.0	38.0
PVP	5.0	2.0	2.0

Physicochemical evaluation of *beras kencur* effervescent

Determination of dissolution time. Dissolution time was measured by dissolving one effervescent tablet (2.5 g) in 50 ml of distilled water (25°C). Time required for the tablet to completely dissolve in water was measured using a stopwatch. It is defined as dissolution time.

Determination of effervescent solution pH. The pH level was measured by dissolving one effervescent tablet (2.5 g) in a beaker containing 50 ml of distilled water (25°C). After completely dissolving, the pH of solution was measured using a pH meter.

Determination of total phenolic content. Following pH measurement, the total phenolic content (TPC) was ascertained using the Folin-Ciocalteau method (Astuti et al., 2023). Effervescent solution (500 µL) was mixed with 1 mL of diluted Folin-Ciocalteau (1:10) (v/v). The solution was incubated at room temperature (25°C) for 8 minutes. Furthermore, 0.8 mL of (Na_2CO_3) solution (7%) was added to the effervescent-folin solution. After 1 hour of incubation, the absorbance of the solution was measured using a UV-vis spectrophotometer (Model Genesys 10 UV, Thermo Scientific, USA) at 765 nm. In this sense, gallic acid, one of the pure and stable natural phenols, was used as standard solution to generate the calibration curve. Gallic acid reacts react with Folin-Ciocalteu reagent to produce a yellow solution, indicating the presence of phenolic. Upon the addition of Na_2CO_3 solution, the -OH group in the phenol compound reacts with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex with an unknown structure that can be detected by a spectrophotometer. At a higher concentration of phenolic compounds, a large number of phenolic ions reduces heteropoly acids to molybdenum-tungsten complexes, resulting in a more intense color (Afrellia et al., 2023). The total phenol content was expressed as mg of gallic acid equivalents per mL of effervescent solution (mg GAE/mL).

Determination of antioxidant activity. The antioxidant activity of the effervescent tablet was measured using Sánchez-Moreno method with some modifications. *Beras kencur* solutions (2500, 5000, 7500, and 10000 mg/L) were prepared by dissolving specific amount of *beras kencur* effervescent in distilled water. Subsequently, 1 mL of each *beras kencur* solution was mixed with 2 mL DPPH 80 mg/L methanol solution. The solutions were then incubated at room temperature (25°C) for 30 minutes. After incubation process, the absorbance of solution was recorded using a UV-Visible spectrophotometer (Model Genesys 10 UV, Thermo Scientific, USA) at 516 nm. The percent of inhibition was calculated according to Eq. (1) as follows (Astuti et al., 2023):

$$\text{Percent inhibition (\%)} = \frac{(A_1 - A_2)}{A_1} \text{ x } 100\% \quad (1)$$

A_1 was defined as the absorbance of DPPH while A_2 was defined as the absorbance of the sample. Thereafter, the linear regression between percent inhibition and sample concentration was performed, and the result was reported as an IC50 value. A lower IC50 value indicates greater DPPH scavenging ability.

RESULT AND DISCUSSION

Physical Characteristics of effervescent

The physicochemical properties of the effervescent tablets studied focused on pH and dissolution time in 50 mL of water at 25°C, with the results presented in Figure 3(a). Formulation F1 has the same pH as the F2 effervescent tablet, likely because the acid-base ratio is not significantly different. Increasing the acid-to-base ratio from 1:1.25 to 1:2.50 increases the pH from 7.5 to 8.0. The decrease in pH with an increasing proportion of citric acid and tartaric acid is attributed to the formation of H^+ ions by the acid. Figure 3(b) shows that tablets F1 and F2 have a long dissolution time (11 seconds). In contrast, the dissolution time of tablet F3 is approximately 2 minutes. It can be concluded that a higher concentration of the base mixture significantly accelerates the dissolution process. Referring to Astuti et al. (2023), which states that the optimum dissolution time for effervescent tablets is less than 10 minutes with a neutral pH value, it can be concluded that F3 is the most optimum formulation.

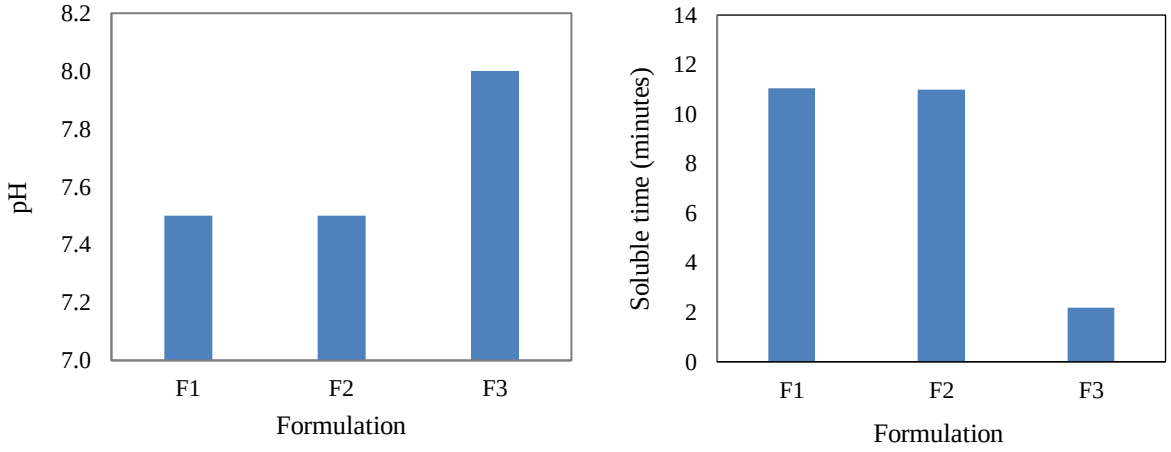

Figure 3. Effect of effervescent formulation to (a) pH and (b) soluble time

In effervescent formulations, the reaction between the acid and base generates carbon dioxide (CO_2), which contributes to the disintegration of the tablet. When the concentration of the base is elevated, it enhances the reaction rate with the acid components, leading to optimal CO_2 production. This is because a greater amount of base facilitates a more efficient neutralization of the acid, thereby accelerating the release of gas. As CO_2 is generated, it creates bubbles that promote rapid disintegration of the tablet matrix, effectively reducing the dissolution time. Additionally, the generation of CO_2 contributes to maintaining a neutral pH in the solution, which is desirable for both stability and palatability. In contrast, formulations with lower base concentrations may exhibit longer dissolution times, as the limited reaction with the acid results in less gas production and a slower breakdown of the tablet. It means the proportions of acids and base affect effervescent solubility. The effervescent solubility is according to the following reaction (Astuti et al., 2023).

$$H_3C_6H_5O_7 \cdot H_2O + 3\ NaHCO_3 \rightarrow Na_3C_6H_5O_7 + 4\ H_2O + 3\ CO_2 \quad (2)$$

$$H_2C_4H_4O_6 + 2\ NaHCO_3 \rightarrow Na_2C_4H_4O_6 + 2\ H_2O + 2\ CO_2 \quad (3)$$

TPC and antioxidant activity

The percent inhibition analysis was conducted to determine the IC50 value, which measures the

sample's potential to inhibit 50% of free radicals (Astuti et al., 2023). Figure 4 shows that the highest inhibition percentage (76.2%) was achieved at a beras kencur concentration of 10 mg/mL. For a serving volume of 150 mL, 1.5 g is required to achieve the highest potential radical scavenging activity. Consequently, 1.5 g of the sample was incorporated into three effervescent tablets.

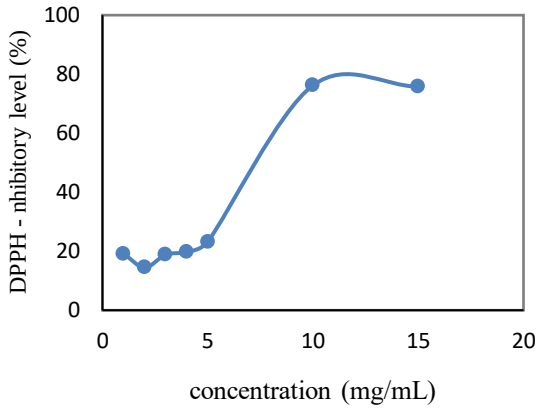

Figure 4. DPPH – percent inhibition of beras kencur

The total phenolic content (TPC) and IC50 values were analyzed to assess the ability of bioactive compounds in the three effervescent formulations to scavenge free radicals, with the results presented in Table 3. The acid-to-base ratio significantly influences the radical-scavenging ability of bioactive compounds, as evidenced by variations in TPC and IC50 values. Generally, low pH enhances the radical-scavenging ability of bioactive compounds due to a higher concentration of H^+ ions. At higher pH, the reduced H^+ ions are replaced by phenoxide ions, which are fully resonance-stabilized and promote greater withdrawal of DPPH electrons. Therefore, a higher IC50 value corresponds to lower antioxidant activity, as shown in Table 4. Under conditions of strong acidity, increased phenol deprotonation can enhance the propensity for autooxidation, particularly in beras kencur formulations with high carbohydrate content. This prooxidant tendency may influence the stability of radical scavenging in the sample. Given that all three formulations exhibit very strong antioxidant activity, F3 is concluded to be the most optimal formulation.

Table 3. Total phenolic content and antioxidant activity

Sample	pH	TPC (mg GAE/mL)	IC_{50} (mg/L)	Antioxidant activity
F1	7.5	10.163 ± 0.07	25.407	very strong
F2	7.5	10.069 ± 0.4	25.174	very strong
F3	8.0	9.799 ± 0.08	24.499	very strong

Table 4. Conversion of IC_{50} Value

IC_{50} (mg/L)	Antioxidant activity
< 50	very strong
51 - 100	strong
101 - 150	moderate
> 150	low

As previously explained, gallic acid, which is one of the pure and stable natural phenols, is used as a standard in determining TPC. The reaction of gallic acid with Folin-Ciocalteu reagent produces a yellow color indicating the presence of phenolic compounds. Gallic acid, due to its chemical structure, demonstrates several mechanisms of action that contribute to its potential in treating breast cancer (Moghtaderi et al., 2018), particularly in the MDA-MB-231 cell model (Figure 4). This compound contributes to the treatment by inducing an increase in nitric oxide (NO-2) production and intracellular reactive oxygen species (ROS) levels (Li et al., 2023). This can result in oxidative stress within cancer cells, leading to their selective damage. GA also enhances apoptosis in breast cancer cells by increasing the population of cells in the sub-G1 phase of the cell cycle, as well as decreasing mitochondrial membrane potential (MMP) and intracellular glutathione (GSH) levels. The prevalence of GSH content in cancer cells plays a significant role in regulating mutagenic mechanisms, DNA synthesis, as well as resistance to multiple drugs and radiation. In malignant tumors, resistance, often correlated with

elevated GSH levels within cancer cells, is notably higher compared to normal tissues (Moghtaderi et al., 2018). This series of events causes mitochondrial dysfunction and release of apoptotic factors, effectively initiating cell death (Singh et al., 2022). Other research showed that gallic acid also enhanced death receptor and p53 signaling, and activation of CASP-3 which is essential for apoptosis in-duction in the MCF-7 breast cancer cell line (Aborehab et al., 2021).

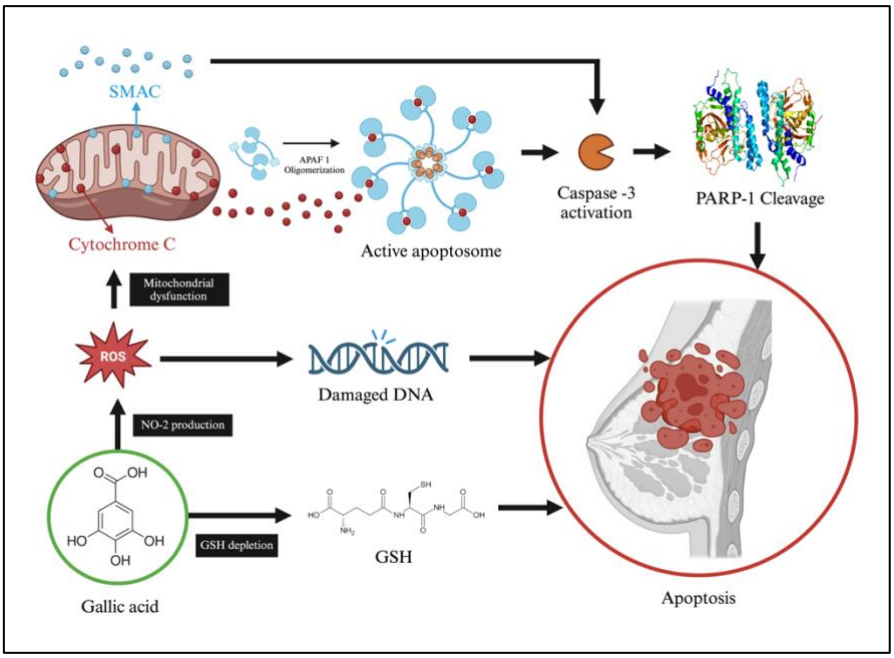

Figure 5. Mechanistic pathway in treating breast cancer (This figure made by BioRender)

CONCLUSION

The ratio of acid and base in the effervescent formulation affects the pH of the solution, which significantly affects the solubility, total phenolic content, and antioxidant activity of effervescent beras kencur. Higher proportion of base (sodium bicarbonate) results in higher solubility. However, higher proportion of acid increases the TPC value and its antioxidant activity.

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