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Effect of heat treatment on antioxidant and antibacterial activity of *Kaempferia galanga L.* extract

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Abstract

Kaempferia galanga L. is a rhizome plant that is rich in health benefits, it can be processed into a herbal drink by heating. Meanwhile, heating is carried out to kill certain pathogenic bacteria present in a product with a minimum loss of nutrients and to maintain the bioactive compounds. Therefore, this study aimed to evaluate the effect of heating using temperatures at 60°C and 80°C for 10, 20, and 30 mins on the antioxidant and antibacterial activity of *K. galanga* extract. The extraction process was carried out using the sonication method with a frequency of 35 kHz for 20 mins and a water-solvent ratio of 1:2 (b/v). The extract was heated at 60°C and 80°C for 10, 20, and 30 mins, and then antioxidant and antibacterial activity were analyzed. The results showed that heating at 60°C and 80°C for 10, 20, and 30 mins significantly decreased the antioxidant and antibacterial activity. Furthermore, the heating using a temperature of 60°C for 10 mins can reduce microbial contamination by 3 logs compared to the control sample and still maintain the total phenolic and flavonoid, antioxidant activity, ferric reducing antioxidant power, ferrous ion chelating, and antibacterial activity of *K. galanga* water extract.

1. Introduction

Kaempferia galanga L. is one of the plant species developed as a widely used commodity due to its bioactive compounds that are beneficial for health. One variety of *K. galanga* is "Gading" with a harvest age of 11 months. The volatile compounds' composition includes pinene, camphene, carvone, benzene, eucalyptol, borneol, methyl cinnamate, pentadecane, and ethyl-p-methoxycinnamate (Tewtrakul *et al*., 2005). It also contains total phenol equivalent to 146 mg gallic acid and 77 mg ascorbic acid equivalent to antioxidant capacity (Chan *et al*., 2008). Furthermore, *K. galanga* is widely used in various types of processed foods such as cooking spices and *K. galanga* herbal drinks which are classified as functional drink because it is rich in bioactive compounds. Generally, the herbal drink is processed by heating at a certain temperature and time. This process kills certain pathogenic bacteria present in the product with a minimum loss of nutrients and maintains moderate physical properties and taste. This study used temperatures of 60°C and 80°C for 10, 20, and 30 mins to evaluate the effect of heating on antioxidant and antibacterial activities.

The heat treatment has a potentially significant effect on the total phenol levels and prolonged heating might destroy phenol compounds in cell components, thereby making its extraction difficult. The oxidation of phenolics is accelerated by the influence of temperature and heating period (Jahangiri *et al*., 2011). Furthermore, the total flavonoid in *K. galanga* water extract decreases along with an increase in extraction temperature. Flavonoids are easily damaged at high temperatures, the decrease in their total content along with increasing temperature occurs due to damage to the cell structure by various chemical reactions that include light and oxygen. Temperatures of 60°C and 80°C were used for 10, 20, and 30 mins in line with Apple cider preservation which currently uses 76 – 87.7°C. Heating at this temperature range causes chemical and nutritional changes in the fruit juice, thereby affecting the quality of the product (Aguilar-Rosas *et al*., 2007). Pomegranate fruit at the heating treatment of 90°C caused 25% degradation of anthocyanin (Vegara *et al*., 2013). Strawberries, cherries and orange jams also caused a decrease in total phenolic content of 12.5% on heating treatment of 45°C (Rababah *et al*., 2011).

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Sonication is one of the extraction methods that utilize waves to speed up the process, it uses a frequency above 20 kHz (Mathialagan *et al*., 2017) and the main advantages include greater efficiency, shorter operating time, and usually a quicker mass transfer rate compared to conventional extraction using soxhlet (Luque-García and Luque De-Castro, 2004). The extraction efficiency is influenced by various factors such as temperature and time, type and concentration of solvent, the ratio of material to solvent, as well as particle size (Chew *et al*., 2011). Furthermore, sonication extraction affects bioactive compounds such as phenols and flavonoids.

Phenolics and their derivatives such as flavonoids are a group of compounds that can capture and stabilize free radicals by donating electrons. They have a broad spectrum of diversity which is distinguished based on the accompanying functional groups. Therefore, differences in functional groups between phenolics and flavonoids cause variations in antioxidant activity. Flavonoid compounds act as antioxidants through the donation of hydrogen atoms or through their ability to chelate metals, this is because flavonoid compounds are a form of glucoside containing glucose side chains or flavonoids in a free form called aglycones (Ndhlala *et al*., 2010).

Aside from bioactive compounds, microbial contamination is important to food safety. Previous studies which investigated the microbiological quality and antioxidant activity of *K. galanga* found that processing without heating produced a shelf-life of approximately 6 hrs, with a total bacteria count (*Escherichia coli*) of 1.6×10^8 CFU/mL in a herbal drink sample at the traditional herbal centre in Kiringan, Bantul, Indonesia. The heating process of *K. galanga* extracts showed that high-temperature treatment reduced the constituent bioactive compounds (Kiptiyah *et al*., 2017).

In this study, antibacterial activity was analyzed using five types of bacteria namely *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar* Typhimurium, and *Streptococcus aureus*. There are no previous studies related to the effect of heat treatment on changes in the total phenolic and flavonoid content, as well as the antioxidant and antibacterial activities of *K. galanga* extract. Therefore, this study aims to evaluate the effect of heat treatment at 60°C and 80°C for 10, 20, and 30 mins in *K. galanga* water extract on total phenolic and flavonoid contents, microbial contamination, as well as antioxidant, and antibacterial activity.

2. Materials and methods

2.1 Materials

The *K. galanga* rhizome was obtained from local farmers in Nogosari, Boyolali, Indonesia, while materials for chemical analysis (analytical grade) were obtained from Merck KGaA, Darmstadt, Germany, these include methanol pa, ethanol pa, NaCl pa, distilled water, stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid (AA= ascorbic acid), gallic acid (GA = gallic acid), quercetin (Q = quercetin), EDTA (ethylenediaminetetraacetic acid), Folin-ciocalteu reagent, Ferrozine (3-(2-pyridyl)-5,6diphenyl-1,2,4-triazine-p, p-disulfonic acid monosodium salt hydrate), catechol, Na_2CO_3 pa, $NaNO_2$ pa, $AlCl_3.6H_2O$ pa, NaOH pa, *2,4,6-tris(2-pyridyl)-s-triazine* (TPTZ), iron (II) sulfate heptahydrate, aluminium chloride, PCA (*Plate Count Agar*), Nutrient broth, Skimmed milk for microbiology, *Chloramphenicol,* as well as a bacterial culture were obtained from culture lab of biotechnology consisting of *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. enterica* ser. Typhimurium, and *S. aureus*.

2.2 Kaempferia galanga extraction

Kaempferia galanga rhizome was cleaned, peeled to ± 3 cm in size, and blanched using hot water at 90°C for 5 mins, then, it was cooled with cold water at 4°C for 5 mins and drained. The blanched sample was crushed using a blender with a water-to-solvent ratio of 1:2 (b/v), and then the extraction process was carried out using the sonication method with a frequency of 35 kHz for 20 mins. The liquid extract was heated at 60°C and 80°C for 10, 20, and 30 mins, then the sample was stored in the refrigerator at 4°C for further analysis. Meanwhile, *K. galanga* water extract without heating was used as a control. The heating process was also carried out at 60°C and 80°C for 10, 20, and 30 mins to evaluate changes in total phenolics and flavonoids, as well as antioxidant and antibacterial activity.

2.3 Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method with gallic acid used as the standard (Kiptiyah *et al*., 2017). The absorbance was measured in a solution of gallic acid with a wavelength (λ) of 750 nm using a double beam UV-VIS spectrophotometer (Thermo Spectronic, Genesys 20*,* USA).

2.4 Determination of total flavonoids

The total flavonoid content was determined by a method based on Aluminum Chloride Colorimetry (Kiptiyah *et al*., 2017). The absorbance of the standard (+)-quercetin solution was measured at a wavelength (λ)

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of 415 nm using a UV-VIS spectrophotometer (Thermo Spectronic, Genesys 20, USA).

2.5 Determination of antioxidant activity using the 2-2-diphenyl-1-picrylhydrazyl method

The antioxidant activity was measured from the free radical scavenging capacity using the 2-2-diphenyl-1-picrylhydrazyl (DPPH) method with modification (Kiptiyah *et al*., 2017). Free radical scavenging power is expressed in % RSA.

$$\%\ RSA = \frac{Absorbance\ blank - Absorbance\ sample}{Absorbance\ blank} \times 100$$

2.6 Ferric reducing antioxidant power analysis

The FRAP method was used to determine the reducing activity of ferrous ions with modification (Gül and Pehlivan, 2018). Antioxidant activity was calculated using a standard curve of $FeSO_4.7H_2O$ and expressed in mmol Fe^{2+}/L of *K. galanga* extract.

2.7 Ferrous ion chelating analysis

The iron ion chelating activity of *K. galanga* was assessed using the modified FIC test method (Chew *et al*., 2009). The formula for calculating the percentage inhibition of complex ferrozine-Fe^{2+}- formation in the *K. galanga* extract is as follows:

$$\%\ ferrous\ ion\ chelating = \frac{1 - absorbance\ sample}{absorbance\ control} \times 100$$

2.8 Total microbes (total plate count)

Approximately 1 mL of the sample was taken and placed into 9 mL of diluent with a level ranging from 10^2 to 10^8. From each dilution, 1 mL was pipetted to sterilize the Petri dishes, then 15-20 mL sterile PCA (Plate Count Agar) media was added, and the Petri dishes were incubated at 37°C for 2 days (48 hrs). The total number of microbes was calculated using the Standard Plate Count (SPC) Harrigan method.

2.9 Antibacterial activity analysis

The antibacterial activity used the agar diffusion method, 200 mL of bacteria culture was added to 20 mL of Nutrient Agar (NA) media. Approximately 75 µL of sample extract was added to the hole and incubated at 37°C for 24 hrs. The diameter of the inhibition zone was observed as antibacterial activity.

2.10 Statistical analysis

The experimental design in this study used a Completely Randomized Design (CRD) with 2 factors, as well as 3 sample and 2 analysis replications. The data obtained were then analyzed statistically using the one-way analysis of variance (ANOVA) method. When there is a difference between treatments, the analysis is continued with Duncan's Multiple Range Test (DMRT) analysis at a significance level of $p < 0.05$.

3. Results and discussion

3.1 Total phenolics and flavonoids content

Certain plants such as seeds, oils, legumes, spices, and teas are known to contain phenolic compounds with antioxidant activity. Bioactive compounds in agricultural products include phenolics which correlate strongly with flavonoids. Meanwhile, flavonoids have biological activities including antiallergic, antiviral, anti-inflammatory, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and most importantly the ability to reduce and scavenge free radicals (Rice-Evans *et al*., 1996).

Figure 1 shows that the heating process at 80°C for 30 mins significantly reduced the total phenolic content of the *K. galanga* water extract to 41.89 mgGAE/L and the total flavonoid to 118 mg QE/L. At 80°C for 10 and 20 mins, the total phenolic was 40.57 and 40.92 mgGAE/L, while the total flavonoid was 150.87 and 124.2 mgQE/L, respectively. This is because the use of high temperatures caused the release of volatile compounds which are bioactive components from the water extract. There were no significant differences between heating for 10 and 20 mins. Meanwhile, at 60°C for 10, 20, and 30 mins, the total phenolic was 50.2, 51.58, and 49.69 mgGAE/L while the total flavonoid was 238.87, 201.87, and 162.53 mg QE/L, respectively. This shows that heating at 60°C for 10 mins slightly reduced the total phenolic content. At a temperature of 60°C for 10 mins, there was no significant decrease compared to the control sample. These results show that prolonged heating at 60° C and 80°C for 30 mins significantly reduced the total flavonoid content compared to 10 and 20 mins.

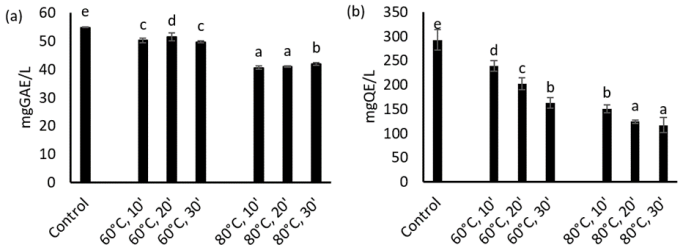

Figure 1. Total phenolic (a) and total flavonoid (b) of *K. galanga* water extract. Bars with different notations are statistically significantly different ($p<0.05$).

Phenolic compounds are known to play a major role in antioxidant activity, the greater the phenolic compounds, the greater the antioxidant activity (Konaté *et al*., 2011). The heating process increased the antioxidant activity of coriander leaves (*Coriandrum*

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sativum L.), but high-temperature thermal processes (>120°C) tend to damage bioactive compounds and reduce antioxidant activity. Heat treatment of coriander leaves either by microwave with 100-900 watts for 960-290 seconds or oven at 40-120°C for 264-48 mins increased the total phenolic and flavonoid, free radical scavenging, and ABTS at 40-80°C, but reduced these parameters at 100-120°C (Hihat *et al*., 2017). Moreover, heating up to 130°C for 5 mins, significantly reduced the antioxidant and phenolic activity of some spice extracts.

In this study, the total phenolic obtained between 60°C and 80°C had a significant decline. This is because the high temperature can damage the bioactive compounds in the sample so that the total phenolic obtained declines. Meanwhile, the heating period of 60°C for 10, 20, and 30 mins tended to be not significantly different. At the temperature of 80°C for 30 mins, it was higher than 10 and 20 mins. This was due to the assumption that at 30 mins of the heating period, new phenolic compounds were formed that were not formed at 10 and 20 mins, the total phenolic at 80°C for 30 mins was slightly higher.

A prolonged heating period reduces the total phenolic levels because it destroys the content in cell components, thereby making its extraction difficult. This is because the oxidation of phenol compounds by atmospheric oxygen is accelerated by the influence of temperature and heating time (Jahangiri *et al*., 2011). Heating at 60°C for 10 mins effectively maintained the bioactive compounds present in *K. galanga* water extract. This heating period also caused a decrease in total phenolic and flavonoids. However, at 80°C for 30 mins, the total phenolic and flavonoid significantly reduced compared to 10 mins. A previous study reported bioactive compound damage due to a high heat treatment in several food commodities as apple juice heated up to 87.7°C had a 32% reduction in the phenolic content compared to the sample processed without heating (Aguilar-Rosas *et al*., 2007).

3.2 Antioxidant activity

Antioxidant activity inhibits free radicals that cause various diseases related to oxidation, such as cardiovascular disease and cancer. A lack of antioxidants in the body leads to weak immunity against free radical attacks (Arivazhagan *et al*., 2000). Free radicals are one or group of atoms that have one or more unpaired electrons, are very reactive, and interact with different body cells. In contrast, antioxidants reduce the negative effects of free radicals. Antioxidant compounds are found in plants and fruits, such as carotenes, flavonoids, and other phenolic components (Teow *et al*., 2007). In addition, other compounds such as vitamins C and E are useful in protecting the human body against the effects of free radicals (Rohman *et al*., 2006). Antioxidants are classified based on the chemical reactions that occur with free radicals into two groups namely the hydrogen atom transfer based test (HAT) and the electron transfer based test (ET) (Dontha, 2016).

Figure 2 shows that antioxidant activity after heating at a temperature of 60°C for 10, 20, and 30 mins was 48.49%, 46.68%, and 45.20%, respectively. This means that the antioxidant activity was maintained and not significantly different from the control sample. Meanwhile, at 80°C for 10, 20, and 30 mins the values were 38.81%, 33.30%, and 21.42%, respectively, indicating that there was a significant decrease in the antioxidant activity compared to heating at 60°C for 10 mins which was 48.49%. The prolonged duration of heating significantly reduced the antioxidant activity at a temperature of 80°C for 30 mins, indicating that the higher the temperature and duration of heating, the higher the decrease in antioxidant activity. Furthermore, DPPH radical scavenging showed that heating up to 80° C for 30 mins caused a decrease in antioxidant activity while heating at temperatures above 80°C led to the loss of the radical scavenging activity. *K. galanga* extract has antioxidant activity as a functional food, while Olive oil is a functional oil with beneficial effects such as antioxidants (Rohman *et al*., 2020).

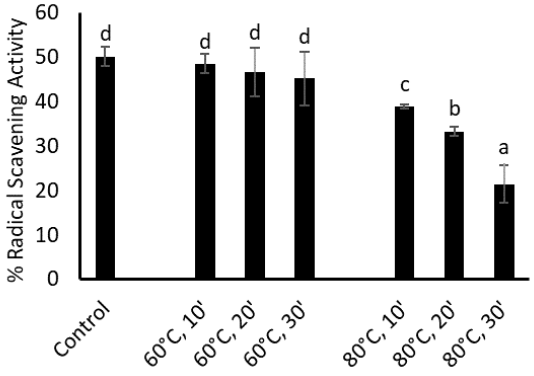

Figure 2. Radical scavenging activity of *K. galanga* water extract. Bars with different notations are statistically significantly different ($p<0.05$).

The total phenolic and flavonoid content has a strong correlation with antioxidant activity, ferric ion reduction, and ferrous ion chelating activity. It results show that prolonged heating at 60°C and 80°C for 30 mins significantly reduced the total flavonoid content compared to 10 and 20 mins in line with the antioxidant activity of *K. galanga* extract. Correlation between total polyphenols and flavonoids with DPPH radical scavenging activity was identified in cacao bean extract but these were not correlated with ferrous ion chelating activity (Indiarto *et al*., 2019). This is because bioactive compounds in agricultural products are in the form of

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phenolic compounds which have strong antioxidative properties, hence, there is a correlation between the two.

3.3 Ferric reducing antioxidant power

FRAP assay is a test that directly measures antioxidant activity in samples compared to other tests that use free radical inhibition. The FRAP method is based on the reduction of the colourless Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ after interacting with the antioxidants present in the sample. This method directly measures the total antioxidants in a food ingredient and has been used to assess the total antioxidants and compare the efficiency of different compounds.

The results showed that the heating temperature between 60°C and 80°C for 10, 20, and 30 mins did not significantly decrease the Fe^{3+} ions reducing power which was 33.39 mmol Fe^{2+}/L – 34.61 mmol Fe^{2+}/L. However, there was a significantly different decrease compared to the control sample, which was 41.55 mmol Fe^{2+}/L as shown in Figure 3. The higher the temperature, the lower the ferric ion reduction ability. In the results of this study, between the temperature of 60°C and 80°C, the frap value was not significantly different, but a significant decline occurred when compared to the control sample (without heating process).

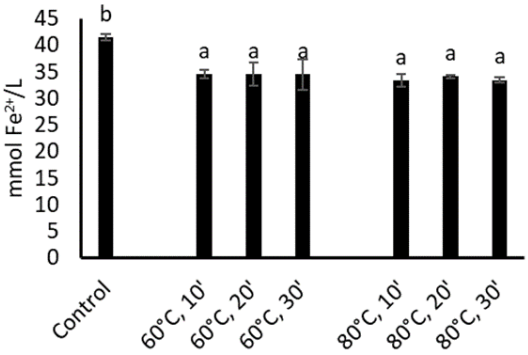

Figure 3. Ferric reducing antioxidant power of *K. galanga* water extract. Bars with different notations are statistically significantly different ($p<0.05$).

The higher the ability to reduce ferric ions, the higher the antioxidant activity. According to (Neoh *et al*., 2016), the reducing ability of seaweed is mostly related to its phenolic content. This is in line with the results obtained in this study where the total phenolic tends to decrease with increasing extraction temperature, hence, the reducing ability also decreases with increasing temperature. A previous study reported that *Eucheuma spinosum* extracted at different temperatures had the same DPPH antioxidant activity compared to the FRAP method. This trend also occurred in two species of red seaweed, *Amansia multifida,* and *Meristiella echinocarpa*, from the northeast coast of Brazil (De Alencar *et al*., 2014).

3.4 Ferrous ion chelating

FIC is a method used to test the ability of a compound to chelate *Fe* metal. This method measures the ability of antioxidants and compounds to compete with ferrozine in chelating iron ions (Elmastaş *et al*., 2006). Ferrozine chelate Fe^{2+} to form complexes that are disturbed by the presence of other metal-chelating compounds. The presence of excessive transition metal ions in the body potentially leads to the formation of Reactive Oxygen Species (ROS) which initiate lipid peroxidation and this process is exacerbated by a metal catalyst. Metal ions also initiate autoxidation by reacting directly with the substrate and/or hydroperoxides present in the system or formed in the propagation step.

The FIC activity with a heating temperature of 60°C for 10, 20, and 30 mins was 54.85%, 58.61%, and 52.58%, respectively. At 60°C for 10 mins, the result was not significantly different from the control, which was 54.85%. However, at 80°C for 30 mins, there was a significant decrease compared to the control, and the sample was at a heating temperature of 60°C, which was 35.81% (Figure 4). The results also indicated that the higher the ferric ion reducing ability, the higher the antioxidant activity. Based on the results, the total phenolic and flavonoid content has a strong correlation with the antioxidant, ferric ion reducing, and FIC activity of *K. galanga*.

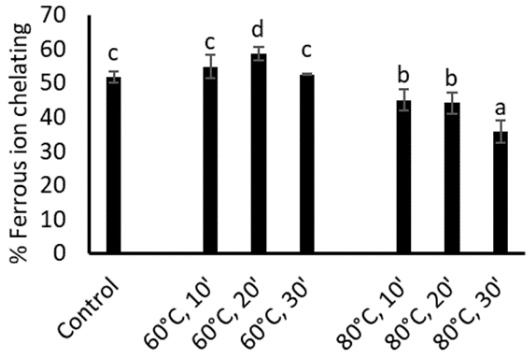

Figure 4. The ferrous ion chelating activity of *K. galanga* water extract. Bars with different notations are statistically significantly different ($p<0.05$).

The metal ion chelating activity parameter indicates the sample's ability to chelate metal ions. If a metal has been chelated, a stable complex will be formed and the oxidation reaction can be inhibited. The correlation between the previous parameters showed that with the decline in total phenolic and flavonoid, the activity of metal ion chelating ability also declined. This is because the correlation between the two is a strong correlation. Chelation is an equilibrium reaction between metal ions and a binding agent, characterized by the formation of more than one bond between the metal and the binding

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agent molecule, which causes the formation of a ring structure. The mechanism of binding Al^{3+} and Fe^{2+} by functional groups of organic components is due to the presence of one carboxyl and one phenolic group, or two adjacent carboxyl groups that react with metal ions. When a metal has been chelated, a stable complex is formed and the catalyzed reaction is inhibited (Berlett *et al*., 2001).

3.5 Microbial contamination

Heating is a thermal process in which food products experience temperature treatment to kill certain vegetative microbes, namely pathogens and inactivating enzymes. However, considering that this process does not kill all vegetative and spore-forming microbes, products are often packaged or stored at low temperatures with the addition of preservatives, and under conditions such as modified atmosphere packaging, pH, or water activity to control microbial growth. Apple cider preservation currently uses a temperature of 76 – 87.7°C, this heating causes chemical and nutritional changes in the fruit juice, thereby affecting the product quality.

Table 1 shows that heating at 80°C for 30 mins and 60°C for 10 mins reduced microbial contamination by 5 logs and 3 logs, respectively, compared to the control sample. Meanwhile, the total microbial contamination allowed for *K. galanga* products is log 4, hence, the heat treatment at 60°C for 10 mins can be categorized as "safe" from contamination. This shows that the use of temperature effectively reduces microbial contamination. Beverage products with low acid content contain several microbes in the form of *E. coli, Salmonella*, *Cryptosporidium* which are pathogenic bacteria that grow when the product is not heated, for example in fruit juice drinks. A common technique of heating fruit juice drinks is to use temperatures that serve to kill or eliminate pathogenic microbes (Canitez, 2002). The heating process at the temperature of 60°C is 10 mins which can be categorized as safe causing decreased microbial contamination and still maintaining the bioactive compounds in *K. galanga* extract.

Table 1. Total plate count of *K. galanga* water extract

Treatment	Total Plate Count (CFU/mL)
Control	1.9×10^{7b}
60°C, 10'	3.7×10^{4a}
60°C, 20'	1.1×10^{4a}
60°C, 30'	4.2×10^{3a}
80°C, 10'	3.2×10^{4a}
80°C, 20'	7.9×10^{4a}
80°C, 30'	6.3×10^{2a}

Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.6 Antibacterial activity

A bacterial growth curve was observed to determine the incubation period of each bacterium used in the antibacterial activity test. As shown in Figure 5, *E. coli*, *B. subtilis*, *P. aeruginosa*, S. *enterica* ser. Typhimurium, and *S. aureus* have different growth rates. The stationary phase was at 24 hrs, hence, the incubation period of the five bacteria was determined at 24 hrs (Figure 5). Antibacterial activity is determined by the content of bioactive compounds contained in the material. The secondary metabolites present in *K. galanga* with antibacterial properties include flavonoids, phenolics or polyphenolic compounds, and alkaloids. Flavonoids are the largest group of phenolic compounds. They function as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane, interrupt the function of microorganism cells, and inhibit the microbial cell cycle. Spices are a potential source of antibacterial agents against some foodborne pathogens and could be suitable as food preservatives (Chakraborty *et al*., 2020).

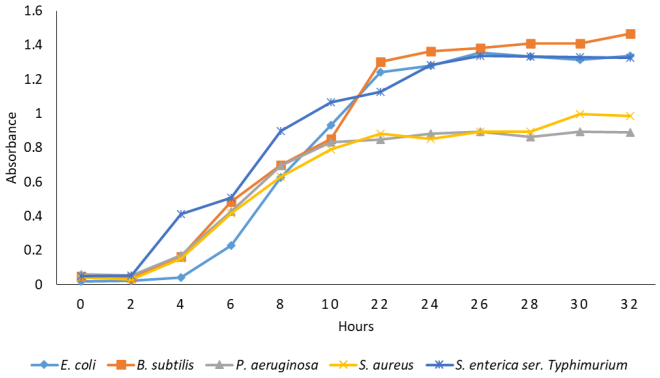

Figure 5. Bacterial growth curve

The *K. galanga* water extract inhibited growth by forming an inhibitory zone on various bacteria, namely *E. coli*, *B. subtilis*, *P. aeruginosa*, S. *enterica* ser. Typhimurium, and *S. aureus* (Table 2). The antibacterial activity ranged from 3 to 9 mm in the zone of inhibition. This is also in line with a similar study which reported that the average antibacterial activity on the inhibition zone was 10 mm. Isolated bioactive compounds had a low effect in inhibiting the growth of S. *enterica ser.* Typhimurium (Fajeriyati and Andika, 2017). Meanwhile, antioxidant properties have a strong correlation with antibacterial activity.

A previous study showed that the use of spices as a substitute for chemicals and synthetics has become indispensable because they have secondary metabolites with antibacterial effects (Nabavi *et al*., 2015). Moreover, Sapodilla fruit stems and juices are inexpensive natural sources of microbes against pathogenic microorganisms, which can overcome the

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Table 2. Antibacterial activity of *K. galanga* water extract based on bacterial inhibition zone

Types of Bacteria		Inhibition zone diameter (mm)						
		Control	60°C, 10'	60°C, 20'	60°C, 30'	80°C, 10'	80°C, 20'	80°C, 30'
Gram +	*S. aureus*	8.89±0.02^{c}	8.73±0.06^{c}	8.69±0.13^{c}	8.85±0.35^{c}	6.99±1.12^{b}	6.36±0.04^{b}	3.66±0.32^{a}
	B. subtilis	8.94±0.73^{d}	8.34±0.15^{cd}	7.14±0.52^{bc}	7.01±0.07^{b}	7.58±0.64^{bc}	6.72±0.77^{ab}	5.49±0.44^{a}
Gram -	*E. coli*	9.65±0.65^{b}	9.47±0.46^{b}	7.53±0.52^{a}	7.35±0.37^{a}	5.92±0.64^{a}	5.82±0.59^{a}	5.79±0.46^{a}
	S. *enterica* ser. Typhimurium	8.04±0.59^{c}	7.59±0.16^{bc}	6.32±0.43^{a}	7.59±0.02^{bc}	7.36±0.13^{bc}	6.71±0.23^{ab}	7.37±0.31^{bc}
	P. aeruginosa	8.92±0.01^{e}	7.36±0.26^{d}	6.75±0.21^{cd}	6.12±0.13^{c}	4.98±0.36^{b}	3.52±0.01^{a}	3.05±0.28^{a}

Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

problem of expensive medical costs (Murnisyazwani and Rabeta, 2019). The difference in the antibacterial activity is presumably due to variations in the structure of the gram-positive and negative bacteria cell walls. Gram-negative bacteria have an outer phospholipid membrane consisting of lipoproteins and lipopolysaccharides that make the cell wall impermeable to plant extracts (El-Chaghaby *et al*., 2014).

Based on the results, heat treatment is important in minimizing microbial contamination and also affects the antioxidant and antibacterial activity of *K. galanga* extract. Meanwhile, the antioxidant and antibacterial properties are also influenced by the total phenolic and flavonoid, the higher the temperature, the greater the tendency to cause damage to these bioactive components. This study shows that 60°C for 10 mins is the most effective temperature in reducing microbial contamination while preserving the phenolic and flavonoid components.

4. Conclusion

Based on the results, the heating process prevented microbial contamination, but the use of a high temperature also reduced the bioactive compound in the extract. The heat treatment at 60°C for 10 mins reduced microbial contamination by 3 logs compared to the control sample but the total phenolic content of 50.2 mgGAE/L, total flavonoids 238.87 mgQE/L, the antioxidant activity of 48.49% RSA, ferric reducing antioxidant power 34.60 Fe^{2+}/L, ferrous ion chelating 54.85%, and antibacterial activity were preserved. In contrast heating at 80°C for 30 mins significantly decreased the antioxidant and antibacterial activity. The antioxidant activity is influenced by the heating process, the higher the temperature used, the lower the antioxidant activity. Furthermore, the antibacterial activity was in the moderate category and influenced by the bioactive compounds present in the extract. The effective treatment chosen was a proper use of heat at 60°C for 10 mins reduced the number of microbial contamination while still maintaining the total phenolic and flavonoid, antioxidant activity, ferric reducing antioxidant power, ferrous ion chelating, and antibacterial activity in *K. galanga* extract.

Conflict of interest

The authors declare that there is no conflict of interest.

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