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Original article

Total phenol content of accessions of Kencur (*Kaempferia galanga* L.) at different altitudes

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Abstract

Kencur (*Kaempferia galanga* L.) is one of the potential medicinal plants with a high enough market demand so that the cultivation of kencur is still quite promising. The purpose of this study was to analyze the effect of altitude on the total phenol content of several accessions of kencur. The research was carried out at the Tropical Biopharmaceutical Laboratory, Bogor Agricultural University, from June to November 2017. The treatment tested was kencur accession rhizome taken from two altitudes, 214 m asl (lowlands) and 780 m asl (highlands). 10 g of kencur accession rhizome powder was added with methanol in a ratio of 1:10, then macerated while shaking using a magnetic stirrer (25 °C; 180 min). The extract was filtered and the filtrate was dried using a rotary vacuum evaporator (50 rpm; 60 °C) until a dry crude extract was obtained. Total phenol was tested by the Folin-Ciocalteu method at a wavelength of 730 nm. The results showed that altitude significantly affected the total phenol content of the kencur accession rhizome extract. The highest total phenol content value was obtained from Galesia 1 (GAL 1) in the lowlands of 17.92 mg/g (equivalent to gallic acid/GAE) and in the highlands obtained from PBG accession (Purbalingga) of 24.85 GAE mg/g compared to other accessions.

INTRODUCTION

Zingiberaceae is a group of plants that are widely used as raw materials for traditional medicines, herbs and spices, food coloring and fabrics, food industry, antimicrobials, and insecticides (Gowda *et al*. 2012; Velayudhan *et al*. 2012; Jan *et al*. 2012; Harit *et al*. 2013; Tripathi *et al*. 2013; Tavares *et al*. 2013). One of the medicinal plants that have the potential to be cultivated from the Zingiberaceae family is kencur (*Kaempferia galanga* L.) because it is a multifunctional plant. Kencur also has good market prospects because it is an important raw material for domestic industries, both for traditional medicines such as treating digestive disorders, respiratory tract and aphrodisiac mixtures, as well as being used for the cosmetics industry based on natural ingredients, so it has the potential to be developed in the downstream sector in the form of extracts, oils, and food or beverage supplements (Badan Penelitian and Pengembangan Pertanian, 2007).

The requirement for plant materials with guaranteed production levels and quality is one step in the process of obtaining high-grade kencur

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products. So far, there are only 3 high yielding varieties of kencur released by the Ministry of Agriculture, namely Galesia 1 (GAL 1), Galesia 2 (GAL 2), and Galesia 3 (GAL 3). The characteristics of the 3 varieties are rhizome production and essential oil content. GAL 3 has the highest productivity (16.2 tons/ha/year) compared to GAL 2 and GAL 1 (15.1 and 14.7 tons/ha/year). Likewise, the essential oil content for GAL 1, GAL 2, and GAL 3 were 3.9%, 6.6%, and 5.6%, respectively. The three varieties are susceptible to *Ralstonia solanacearum* bacteria, and grow well in low, medium, and highlands (Rostiana and Effendi, 2007). Differences in altitude will affect the growth, yield, and quality of kencur.

The quality of kencur is determined by the content of essential oils with the main component ethyl-p-methoxy cinnamate (EPMS), which is a secondary metabolite derived from cinnamic acid from the phenol group which is responsible for the distinctive aroma and taste of kencur (Tripathi *et al*. 2013). Research on the effect of altitude on the secondary metabolite content of kencur has been reported by Roemantyo and Somaatmadja (1996), Yusron and Januwati (2003), Rostiana and Effendi (2007), Rostiana *et al*. (2009), and Rostiana and Subaryanti (2010). However, research on the total phenolic content of several accessions of kencur from Purbalingga (PBG), Cilacap (CLP), Purworejo (PWJ), Karanganyar (KRA), Pacitan (PCT), and Madiun (MAD) at several altitudes has not been carried out. This study aimed to analyze the effect of altitude on the total phenol content of several accessions of kencur.

MATERIALS AND METHODS

The materials used were kencur rhizome aged 6 months from accessions of Purbalingga (PBG), Cilacap (CLP), Purworejo (PWJ), Karanganyar (KRA), Pacitan (PCT), and Madiun (MAD), as well as comparison varieties Galesia 1 (GAL 1), Galesia 2 (GAL 2), and Galesia 3 (GAL 3). Kencur samples were obtained from planting at different altitudes, namely 214 m asl and 780 m asl.

The solvents and chemicals used were methanol p.a, gallic acid p.a, iron (III) chloride ($FeCl_3$), 96% ethanol, Folin-Ciocalteu reagent, sodium hydroxide (NaOH p.a), and aquabidestylates. The instruments used are Rotary Vacuum Evaporator (Buchi R-114, Germany), and U-2800 UV-Vis spectrophotometry (Hitachi, Tokyo, Japan).

The design used was a split-plot design with 3 replications. As the main plot is the altitudes and the accession of kencur as a sub-plot. The altitude consists of 2 levels, namely 214 m asl (lowlands) and 780 m asl (highlands).

Preparation and Extraction of Kencur Rhizome

Samples of 1 kg of rhizomes were taken from each altitude (214 m asl and 780 m asl) simultaneously 6 months after planting (MAP), after cleaning, then thinly sliced and dried in an oven at 40–50oC until the water content was less than 10% ie if the rhizome can be broken then drying is considered sufficient. The dried samples were ground to a powder with a size of 100 mesh (Rostiana *et al*. 2009). 10 g of kencur rhizome powder was added with methanol in a ratio of 1:10, then macerated while shaking using a magnetic stirrer (25oC; 180 min). Next, the extract was filtered and the filtrate was dried using a rotary vacuum evaporator (50 rpm; 60oC) to obtain a dry crude extract (Direktorat Jenderal Bina Kefarmasian and Alat Kesehatan. 2011). Yield was calculated by comparing the weight of the extract with the weight of the material multiplied by 100%.

Identification of Phenol Compounds

One gram of kencur extract was added with ethanol, then shaken until dissolved. Then 2 drops of $FeCl_3$ was added, the formation of a strong green, red, purple, blue, or black color indicating the presence of phenolic compounds in the sample (Harborne, 1987).

Determination of Total Phenol Content

Determination of total phenol content refers to the Indonesian Herbal Pharmacopoeia (Direktorat Jenderal Bina Kefarmasian and Alat Kesehatan 2011). A total of 1 mL of kencur extract with a concentration of 10 mg/L was mixed with 5 mL of Folin-Ciocalteu reagent 7.5% and 4 mL of NaOH with a concentration of 1%, then incubated (25oC; 60 min). The absorbance of the solution was measured using a spectrophotometer at a wavelength of 730 nm. The total phenol of kencur rhizome extract was expressed as milligrams (mg) of gallic acid equivalent per gram of dry extract weight (GAE mg/g of kencur rhizome extract). Gallic acid was used as standard at various concentrations (10, 20, 30, 50, 70, and 100 mg/L).

Data Analysis

To determine the effect of altitude on the total phenol content of kencur rhizome extract, one-way analysis of variance was carried out and followed by Duncan's Multiple Range Test (DMRT) at 5% level. The analysis was carried out with the help of the SPSS version 21.0 program.

RESULTS AND DISCUSSION

Extract of Kencur Rhizome

The GAL 2 kencur rhizome extract were significantly higher (3.44%) than other accessions in the lowlands, meanwhile in the highlands MAD accessions were significantly higher (3.78%) than other accessions, but not different from accessions PCT (3.18%) (Table 1). This indicates that the chemical components found in the lowlands are mostly obtained from GAL 2. It is known that GAL 2 is a high yielding kencur variety that is very responsive and more stable to differences in its growing environment. Kencur GAL 2 is also specifically adapted to the growing environment at an altitude of 350–650 m asl with an essential oil content of 2.1–6.6% (Rostiana *et al*. 2006; Rostiana and Effendi, 2007).

Table 1. The yield of kencur rhizome extract at different altitudes

No.	Accessions	Extract Yield (%)	
		Lowland	Highland
1.	PBG	0.62 [a]	2.91 [b]
2.	CLP	0.56 [a]	2.24 [b]
3.	PWJ	0.64 [a]	1.51 [a]
4.	KRA	1.23 [b]	1.21 [a]
5.	PCT	0.48 [a]	3.18 [c]
6.	MAD	1.21 [b]	3.78 [c]
7.	GAL 1	1.04 [b]	1.06 [a]
8.	GAL 2	3.44 [c]	1.31 [a]
9.	GAL 3	0.52 [a]	1.89 [a]

PBG: Purbalingga, CLP: Cilacap, PWJ: Purworejo, KRA: Karanganyar, PCT: Pacitan, MAD: Madiun, GAL1: Galesia 1, GAL2: Galesia 2, and GAL3: Galesia 3. Numbers followed by the same letters are not significantly different according to Duncan's test at 5% levels.

In the highlands, the most chemical components were obtained from MAD accessions (3.78%). The MAD accession came from Madiun Regency, East Java with an altitude of 600 m asl, it is suspected that genetically the accession had adapted to the growing environment in the highlands (780 m asl) as indicated by the high extract yield. Several factors affecting the extract yield were soil fertility, water availability, genetic variation, growing environment, and geographical conditions (Rostiana and Subaryanti, 2010; Aragaw *et al*. 2011; Kavitha and Menon, 2013; Bermawie et al. 2013; da Costa *et al*. 2014; Preetha *et al*. 2016).

The difference in yield was also caused by the content of bioactive compounds in the sample and showed levels of secondary metabolites carried by the solvent (Ukieyanna, 2012). Methanol is a universal solvent and is able to extract all chemical components found in plants, both non-polar, semi-polar and polar. Methanol is a water-soluble compound that easily enters the cell through the cell wall material, so that the secondary metabolites contained in the cytoplasm will be dissolved in the solvent and the compound will be extracted completely (Verdiana *et al*. 2018). The yield value can be used to estimate the number of bioactive compounds contained, but cannot be used to determine the type of bioactive components (Martono *et al*. 2016).

Identification of Phenol Compounds

As shown in Table 3, the extracts of kencur rhizome from all accessions positively contained phenolic compounds both from the lowlands and highlands. This is indicated by the presence of black color with the addition of $FeCl_3$ and yellow color with Folin-Ciocalteu reagent.

Identification of phenolic compounds is a qualitative test to prove and ensure that samples of kencur extract contain the phenolic compounds. Basically, phenolic compounds are easily soluble in polar solvents such as water, ethanol, and methanol (Harborne, 1987). The reaction of $FeCl_3$ with the sample gives black, violet, green, and blue colors where Fe_3^+ ions play a role in the hybridization process as shown in Figure 1.

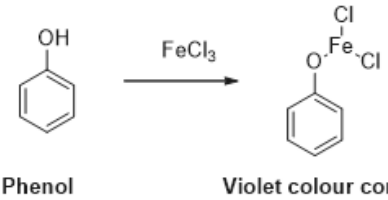

Figure 1. Reaction of phenol with $FeCl_3$ (Sagar, 1996)

Total Phenol Content

The results of the analysis of variance showed that the altitude had a significant effect on the total phenol content. The test results can be seen in Table

3, which shows that the GAL 1 kencur rhizome extract had a total phenol content of 17.92 GAE mg/g significantly higher than that other accessions in the lowlands, while in the highlands the PBG accession had a total phenol content of 24.85 GAE mg/g significantly higher than other accessions.

Table 2. Identification of phenolic compounds of kencur rhizome extract at different altitudes

Access-ions	Lowland		Highland		Results
	$FeCl_3$	*Folin-Ciocalteu*	$FeCl_3$	*Folin-Ciocalteu*	
PBG	Black	Yellow	Black	Yellow	+
CLP	Black	Yellow	Black	Yellow	+
PWJ	Black	Yellow	Black	Yellow	+
KRA	Black	Yellow	Black	Yellow	+
PCT	Black	Yellow	Black	Yellow	+
MAD	Black	Yellow	Black	Yellow	+
GAL1	Black	Yellow	Black	Yellow	+
GAL2	Black	Yellow	Black	Yellow	+
GAL3	Black	Yellow	Black	Yellow	+

+ contains phenolic compounds, PBG: Purbalingga, CLP: Cilacap, PWJ: Purworejo, KRA: Karanganyar, PCT: Pacitan, MAD: Madiun, GAL1: Galesia 1, GAL2: Galesia 2, and GAL3: Galesia 3.

Figure 2. The Reaction Between Gallic Acid and Folin-Ciocalteu reagent. A. Before adding NaOH, B. After adding NaOH

The Galesia 1 kencur variety (GAL 1) is a landras from the Cileungsi population, Bogor, West Java with an essential oil content of 2.08–3.92%, specifically adapted to the growing environment at an altitude of 80–650 m asl and responsive to fertilization (Rostiana *et al*. 2006; Rostiana and Effendi, 2007). The adaptability of PBG accession in the highlands is shown by its ability to increase the phenol content of the rhizome. The PBG accession is the accession of kencur originating from Purbalingga Regency, Central Java from an altitude of 100 m asl. To overcome these environmental differences, this accession is thought to increase phenol compounds as a defense mechanism against environmental stresses, fungal pathogens, insects, and herbivores. The higher the phenol content, the higher the plant resistance is expected (Bermawie *et al*. 2013). Another issue is the relationship between air temperature and total phenol content. Up until a particular degree, an increase in air temperature induces an increase in the phenol content, which then decreases as the temperature rises (Liyana and Shahidi, 2005).

Table 3. Total phenol content of kencur rhizome extract at different altitudes

Accessions	Total phenol content (GAE mg/g)	
	Lowland	Highland
PBG	6.63 ± 0.85 ^{a}	24.85 ± 2,76 ^{j}
CLP	11.99 ± 0.30 ^{cd}	16.61 ± 1.85 ^{gh}
PWJ	9.29 ± 0.09 ^{b}	16.93 ± 0.84 ^{gh}
KRA	12.85 ± 0.09 ^{cde}	23.11 ± 1.52 ^{i}
PCT	13.50 ± 0.69 ^{de}	11.09 ± 0.44 ^{c}
MAD	11.15 ± 0.53 ^{c}	8.65 ± 0.04 ^{b}
GAL 1	17.92 ± 0.45 ^{h}	12.75 ± 0.11 ^{cde}
GAL 2	9.26 ± 0.18 ^{b}	15.37 ± 0.47 ^{fg}
GAL 3	14.22 ± 0.39 ^{ef}	11.98 ± 0.40 ^{cd}

GAE: gallic acid equivalent, PBG: Purbalingga, CLP: Cilacap, PWJ: Purworejo, KRA: Karanganyar, PCT: Pacitan, MAD: Madiun, GAL1: Galesia 1, GAL2: Galesia 2, and GAL3: Galesia 3. Numbers followed by the same letter were not significantly different based on Duncan's test at the 5% level.

As a standard, gallic acid is used which is one of the pure and stable natural phenols. According to Viranda (2009), gallic acid is a phenolic compound derived from hydroxybenzoic acid which is classified as a simple phenolic acid. Gallic acid was reacted with Folin-Ciocalteu reagent to produce a yellow color (Figure 2A) which indicated the presence of phenolic compounds, after which NaOH solution was added to provide an alkaline condition. During the reaction, the hydroxyl group on the phenol compound reacts with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex (Figure 2B) with an unknown structure and can be detected by a spectrophotometer. If there is a higher concentration of phenolic compounds present, then more phenolic ions will reduce heteropoly acid (phosphomolybdate-phosphotungstic) to a molybdenum-tungsten complex, resulting in a more concentrated color. The blue color formed will be more concentrated, equivalent to the concentration of phenol ions formed (Viranda, 2009).

CONCLUSION

The altitudes significantly affected the total phenol content of the kencur accession rhizome extract. The highest total phenol content value was obtained from Galesia 1 (GAL 1) in the lowlands of 17.92 GAE mg/g and in the highlands obtained from the accession of PBG (Purbalingga) of 24.85 GAE mg/g.

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