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Original Article

THE DETERMINATION OF ETHYL *P*-METHOXY CINNAMATE IN *KAEMPFERIA GALANGA* L. RHIZOME EXTRACT HARVESTED IN RAINY AND DRY SEASONS

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ABSTRACT

Objective: *Kaempferia galanga* L. rhizome (KGR), has been empirically used in Indonesia, particularly by Javanese, to cure inflammation. KGR contains various secondary metabolites which explain its pharmacology activities, among them is ethyl *p*-methoxycinnamate (EPMC). However, due to the different seasons of our country, the yield of extraction is often unalike. In this work, we determined the percentage of yield (w/w), the water content (thermogravimetric method), and the concentration of EPMC in the Ethanol extract of *Kaempferia galanga* L. Rhizome (EEKG) harvested from the rainy (EEKG-R) and dry seasons (EEKG-D).

Methods: The sun-dried rhizomes were cold macerated for 3x24 h with 70% ethanol, filtered, and the solvent was evaporated at 40-45 °C until a viscous extract was obtained. The determination of EPMC in the extract was carried out using the RP-HPLC standard addition method. Detection was set at 308 nm; injection volume 20 µl; flow rate 1.0 ml/min. The column used is C18 (length 250 mm, internal diameter 4.6 mm, particle size 5 µm).

Results: The yield of EEKG-R (harvested in the rainy season) = 14.56% w/w, water content = 4.37%, and the EPMC = 0.01%. Meanwhile the yield of EEKG-D (harvested in the dry season) = 5.79% w/w, water content = 18.76%, and the EPMC = 0.001%.

Conclusion: Different climates affect the percentage yield and the quality of the extract. In our work, the EEKG-R (harvested in the rainy season) revealed a better quality compared to that of EEKG-D (harvested in the dry season) This study gives important information to standardize and optimize the harvest time of KG rhizomes for drugs development, which are strongly influenced by seasonal differences.

Keywords: Anti-inflammation, Ethyl *p*-methoxycinnamate, Herbal medicine, *Kaempferia galanga* L

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INTRODUCTION

Recently, the development of herbal-based drugs has become the focus of interest of many academicians and researchers. These drugs are proposed to overcome the problem of unexpected side effects by synthetic drugs. *Kaempferia galanga* L. (KG) rhizome, has been empirically used in Indonesia, particularly by Javanese, to relieve pain, swelling, and other inflammation [1-3].

Researches using the *in vitro* and the *in silico* technique has proven that infusion [4], methanol extract [5], ethanol extract [6, 7], petroleum extract [8], and ethyl *para*-methoxycinnamate (EPMC) isolated from KG rhizome [9, 10], possess an anti-inflammatory effect. EPMC, ethyl cinnamate, propanoic acid, and pentadecane are secondary metabolites contained in the essential oil of the KG rhizome. These secondary metabolites are believed to play a role in their anti-inflammatory activity [2].

Secondary metabolites in plants are part of a mechanism or self-defense system against stress, including stress from the environment due to seasonal differences, intending to preserve the species, maintain plant immunity, and fighting pathogens that endanger its survival [11, 12]. This is the basis for the exploration and development of medicinal plants for humans, by utilizing the secondary metabolites in these plants. As a consequence, an effort to standardize plant raw materials that can produce optimal and uniform secondary metabolites is required. Among the standardization efforts are determining the yield percentage, the water content, and the major secondary metabolite. A method that has been widely used and well-validated is the HPLC system [13-15].

In this work, we determined and compared the percentage of yield (w/w), the water content (thermogravimetric method), and the concentration of EPMC in KG rhizomes harvested from the rainy and dry seasons. The results of this study will add important information to standardize and optimize the harvest time of KG rhizomes for drug development, which are strongly influenced by seasonal differences.

MATERIALS AND METHODS

Materials

The plant material used in this study was the ethanol extract prepared from the KG rhizome. The rhizomes were planted in Buniayu village, Jalan Cagak sub-district, Subang, West Java, Indonesia (6 °37'54.7"S 107 °41'59.2"E). The air temperature in the Jalan Cagak sub-district, Subang, ranges at 21-31 °C, humidity levels range from 78-84%, and the average rainfall>4000 mm [16]. The harvested plant ages range from 8-9 mo. We obtained two harvesting times of the plants, which were in the rainy season (December) and in the dry season (June). The plant samples have been collected and certified taxonomically as previously described [17].

Chemicals used in the extract preparation and phytochemicals screening were 70% ethanol technical grade (Indokimia®, Indonesia), ferric chloride solution (1-3% in distilled water, Merck®, Germany, CAS No. 10025-77-1), sodium hydroxide solution (1-2 N in distilled water, Merck®, Germany, CAS No. 1310-73-2), lead acetate solution (0.5 N in ethanol, Merck®, Germany, CAS No. 6080-56-4), sulfuric acid (Merck®, Germany, CAS No. 7664-93-9), Magnesium (Merck®, Germany, CAS No. 7439-95-4), Dragendorff reagent (Merck®, Germany, CAS No. 39775-75-2), and acetic acid (Merck®, Germany, CAS No. 64-19-7). Chemicals used in the HPLC analysis were ethanol absolute analytical grade (Merck®, CAT No. 1.11727.2500), acetonitrile (Merck®, CAT No. 1.00030.4000), methanol with HPLC grade (Merck®, CAT No. 1.06007.4000), double-distilled water (API IPHA®, Indonesia), and pure EPMC (Tokyo Chemical Industry Co., Ltd., CAS RN: 24393-56-4) as the internal standard. Other supporting materials used were membrane

filters PTFE for mobile phase (pore size=0.45µm, Hawach Scientific®, Item code: SLPT5045SLG) and Whatman™ filter papers (No. 1/120 mm and No.41/90 mm).

Methods

Preparation of the extract

The rhizome was thinly sliced and dried in a thermostatic oven (EHRET) at 50 °C. The KG rhizome extract was prepared by soaking the dried rhizome for 3x24 h in 70% ethanol. The collected liquid was filtered, and the filtrate was evaporated using a rotary evaporator followed by using a water bath (40-45 °C) to viscosity [17].

Calculation of the yield percentage and the water content

The yield percentage was measured by the formula: weight of extract divided by the weight of the macerated dry powder of KG rhizome, multiplied by 100 percent. Meanwhile, the water content is measured by weighing the extract after it was dried subsequently in an oven (Thermo scientific, OGH-100), by following the thermogravimetric method [18].

Phytochemical screening

The detected phytochemical content consisted of polyphenols, flavonoids, alkaloids, tannins, triterpenoids, and saponins. The phytochemical screening method was carried out by following the standard color test [19].

HPLC Analysis to determine EPMC in the ethanol extract of KG rhizome

HPLC analysis to determine EPMC in the ethanol extract of KG rhizome was carried out by following the optimum analytical procedure of Mukkasombut and colleagues (2020) [13],and Wahyuni and co-workers (2021) [17]. The extract samples are EEKG-R (harvested in the rainy season) and EEKG-D (harvested in the dry season). A standard EPMC solution in increased concentrations (40, 20, 10, 5, and 0 ppm for EEKG-R and 3.2, 1.6, 0.8, 0.4, and 0 ppm for EEKG-D) was spiked to each sample. The pure EPMC (Tokyo Chemical Industry Co., Ltd., CAS RN: 24393-56-4) used as an internal standard is certified and purchased from a reputable chemical supplier. The determination of EPMC in the extract was carried out using the RP-HPLC standard addition method. Detection was set at 308 nm; injection volume 20 µl; flow rate 1.0 ml/min. The column used is C18 (length 250 mm, internal diameter 4.6 mm, particle size 5 µm). The mobile phase used was water and acetonitrile with a ratio of 40:60, in isocratic elution.

RESULTS

Table 1 shows the measurement results and the comparison between the yield percentage and water content on the EEKG-R and EEKG-D. Table 1 also shows the results of phytochemical screening results.

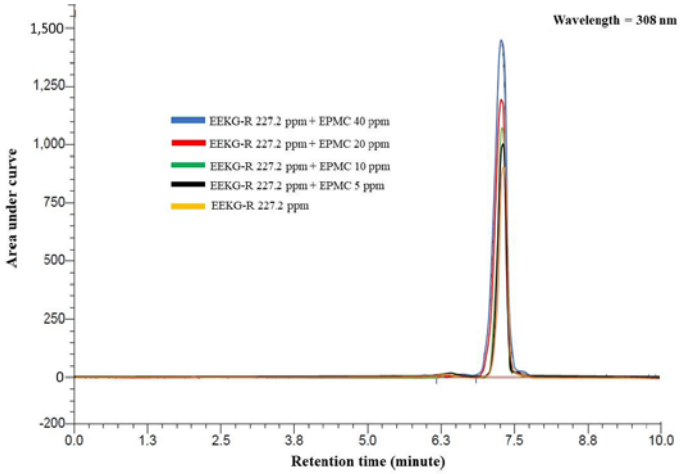

Fig. 1: Chromatogram of EEKG-R (yellow) and EEKG-R spiked with Increased Concentrations of EPMC Standard (other colors) Indicates the Presence of EPMC at tR = 7.28 min

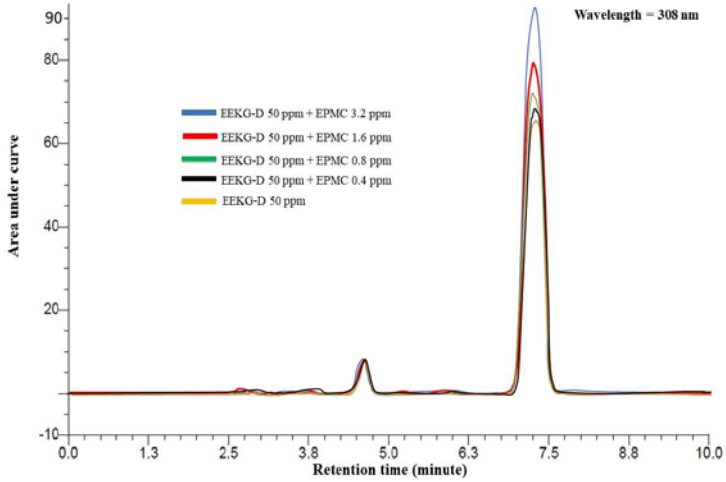

Fig. 2: Chromatogram of EEKG-D (yellow) and EEKG-D spiked with increased concentrations of EPMC standard (other colors) indicates the presence of EPMC at tR = 7.29 min

Table 1: Comparison of yield percentage, water content, and phytochemical content of EEKG-R and EEKG-D

	EEKG-R	EEKG-D
% yield (w/w)	14.56	5.79
Water content (%)	4.37	18.76
Polyphenols	Detected	Detected
Flavonoids	Detected	Detected
Alkaloids	Detected	Detected
Tannins	Detected	Detected
Triterpenoids	Detected	Detected
Saponin	Detected	Detected

Fig. 1 and fig. 2 depict the chromatograms produced from the EPMC analysis in EEKG-R and EEKG-D, respectively. The relative area under the curve (AUC) of EEKG-R and EEKG-D is presented in table 2.

Table 2: HPLC data of EEKG-R and EEKG-D

No	The retention time of EEKG-R (minutes)	The peak height of EEKG-R (mAU)	The relative area of EEKG-R (%)	The retention time of EEKG-D (min)	The peak height of EEKG-D (mAU)	The relative area of EEKG-D (%)
S1	7.25	1444.69	98.85	7.28	92.21	91.73
	7.25	1449.55	97.64	7.29	92.92	92.15
S2	7.27	1169.71	97.39	7.28	79.40	91.93
	7.28	1166.52	97.88	7.28	79.88	90.28
S3	7.28	1066.72	97.12	7.29	73.90	90.32
	7.29	1069.08	96.85	7.29	73.67	90.40
S4	7.29	1018.13	97.23	7.29	69.72	88.66
	7.29	1018.85	96.58	7.29	69.95	88.73
S5	7.29	939.34	97.92	7.31	65.93	89.74
	7.29	942.15	96.39	7.30	66.61	90.51

The standard addition HPLC method shows good linearity as confirmed by the coefficient of correlation (r) value approaching 1 (table 3). The EPMC level in the extracts is presented in table 3.

Table 3: The determination of EPMC in the EEKG-R and EEKG-D

	EEKG-R	EEKG-D
Linear regression equation and coefficient of correlation (r)	y = 2.873x+244.94 r = 0.9970	y = 2.5958x+23.344 r = 0.9930
EPMC level	85.26 µg/ml or 0.01% (w/v)	8.47 µg/ml or 0.001% (w/v)
LOD	7.57 µg/ml	0.99 µg/ml
LOQ	25.24 µg/ml	3.29 µg/ml

DISCUSSION

Polyphenols, flavonoids, alkaloids, tannins, triterpenoids, and saponin were detected in both EEKG-R and EEKG-D (table 1). Our previous study confirmed that polyphenols were contained in the extracts harvested in the rainy season, using thin-layer chromatography (TLC), spectrophotometry, and HPLC [17].

The percentage yield of EEKG-R is higher than that of EEKG-D, whereas the water content of EEKG-R is lower than that of EEKG-D (table 1). The optimum requirement for the synthesizing of extracts from *Kaempferia galanga* L. rhizome is to produce yield>8% and water content<10% [20]. Several studies stated that the synthesis of extracts of *Kaempferia galanga* L. rhizome using the cold maceration method with 96% ethanol resulted in varying yields of 4% [8], 5.86% [7], 12.67% [21], and 20.56% [22]. Other studies confirmed that EPMC had been isolated from the active sub-fraction of a chloroform extract KG (yield = 0.026%) [9]. However, none reported the comparison between yield, water content, and the concentration of EPMC in KG rhizomes harvested from different climates.

The difference in the yield percentage and the water content proved that climate is important in affecting the yield. The best planting time for KG is at the beginning of the rainy season and harvesting is usually done after 11 mo at the next rainy season [21]. During the dry season, the plants get access to the water from deeper sources, while in the rainy season most of the water is obtained from the upper soil layers. Thus, in the rainy season, shallow lateral roots or rhizomes remain well-hydrated [23, 24].

The chromatogram peaks of EEKG-R and EEKG-D, detected at 308 nm, indicating that EPMC is positively contained in both extracts and is eluted at 7.2 min. The rhizome harvested in the rainy season resulted in a higher EPMC level than that of the dry season. The EEKG-R shows better quality than the EEKG-D, thus the best harvesting time for KG is the rainy season.

There are various methods of extracting KG rhizome, e. g. maceration using ethanol [7, 8, 17, 21, 22, 26, 27], maceration using dichloromethane [28], hydro-distillation at 100–105 °C [29], serial extractions using petroleum ether, chloroform, methanol, and water [9, 10], etc, however, the level of EPMC reported by previous authors is not unalike, which is very low and in line with our result.

CONCLUSION

Different climates affect the percentage yield and the quality of the extract. In our work the EEKG-R (harvested in the rainy season) revealed a better quality (yield = 14.56% w/w, water content = 4.37%, EPMC = 0.01%) compared to that of EEKG-D (harvested in the dry season) (yield = 5.79% w/w, water content = 18.76%, EPMC = 0.001%). This study gives important information to standardize and optimize the harvest time of KG rhizomes for drugs development, which are strongly influenced by seasonal differences.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

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