Analysis of bioactive compounds present in *Kaempferia galanga* rhizome collected from different regions of East Java, Indonesia

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**Abstract.** *Kaempferia galanga*, also known as kencur in Indonesia, is a medical plant that is traditionally used in various human diseases therapy. Ethyl p-methoxycinnamate (EPMC) is an essential bioactive compound in kencur that plays a role in the management of diseases. Kencur is widely spread in East Java. Thus this study aims to identify and analyze the bioactive compounds in kencur rhizome from a different region in East Java. This study was conducted from June 2020 until November 2020. The samples were collected from 12 regencies in East Java, including Banyuwangi, Blitar, Gresik, Kediri, Lumajang, Madura, Malang, Mojokerto, Nganjuk, Pacitan, Ponorogo, and Trenggalek. A sample from Wonogiri (Central Java) was used as a comparison because its cultivation procedure has been standardized. The altitude of sample accessions location was 15-995 masl (m above sea levels) with the rain intensity of 500-2000 mm⁻¹. Based on soil analysis, Banyuwangi had good soil characteristics with high total nitrogen, available P₂O₅, cation exchange capacity (CEC), and organic carbon. The bioactive compounds were analyzed using gas chromatography-mass spectrometry (GC-MS). The results showed that all the samples contain three major compounds: EPMC, pentadecane, and (Z)-ethyl cinnamate. The highest content of EPMC was obtained in Wonogiri (79.8%), followed by Malang (78.28%), Blitar (77.23%), and Trenggalek (73.33%). It can be concluded that the kencur samples from Malang, Blitar, and Trenggalek have a high content of EPMC, thus could become the source of kencur used in further medicinal research.

1. Introduction
Medicinal plants have been widely used for medicinal purposes because it naturally contains bioactive compounds that can prevent or treat various diseases. Plants from the Zingiberaceae family are commonly used in Jamu, Indonesian traditional herbal medicine, such as *Curcuma domestica*, *Curcuma xantorrhiza*, *Curcuma heynaeana*, *Zingiber officinale*, and *Kaempferia galanga* [1]. Kencur (*Kaempferia galanga*) belongs to the Zingiberaceae family and its rhizome has been used traditionally in the treatment of various diseases, such as hypertension, diabetes, and pain management. The kencur rhizome contains 49 compounds, such as terpenoids, flavonoids, ester, phenolic acids, and esters as well as rich in bioactive essential oil. The major constituents that were found in kencur oils from different origin were ethyl p-methoxycinnamate, ethyl cinnamate, 3-carene, pentadecane, borneol, bornyl acetate, δ-selinene, camphor, and α-pinene [2]. It has many biology activities, such as anti-cancer, anti-microbial, anti-
oxidant, anti-inflammatory, anti-nociceptive, wound healing, and has effects on vascular functions [3]. Based on Indonesian Traditional Medicine Formularium, kencur can be used for migraines, sprain, fatigue, and arthritis [4].

East Java is the centre of kencur rhizome production in Indonesia, with a total of 8.759 tons per year in 2018 based on data from the Indonesian Central Bureau of Statistics (Badan Pusat Statistik) [5]. Every region has different characteristics of kencur, which depend on the environment and agronomic character. The aim of this study was to analyze the bioactive compounds in kencur rhizome from different regions in East Java and analyze the environmental characteristic (climate and soil condition) of each cultivation location.

2. Materials and method
This research was started from June 2020 until November 2020. The location of the study was randomly selected (Figure 1). The plant samples were collected from 12 regencies in East Java including Banyuwangi, Blitar, Gresik, Lumajang, Madura, Mojokerto, Malang, Nganjuk, Pacitan, Ponorogo, and Trenggalek. Sample from Wonogiri was also collected as comparator regency (standardized place for kencur cultivation in Indonesia). The altitude for each sampling area was recorded using GPS, and the light intensity was determined using Luxmeter. The temperature, humidity, and rainfall data of each location were collected from the Central Bureau of Statistics in every regency.

In this study, we also analyzed the soil condition of each location. Soil sample (0 to 30 depth) was taken from five randomly selected plots in each sampling site. The five soil samples were combined into a single 1000 g composite sample. The composite samples of each location were analyzed at the Assessment Institute for Agricultural Technology East Java, Indonesian Agency for Agriculture Research and Development, Ministry of Agriculture, Malang, Indonesia. Water content was determined by gravimetric method, total nitrogen was determined by Kjeldahl method, available $\text{P}_2\text{O}_5$ was determined by Olsen method, cation exchange capacity (CEC) was determined using atomic absorption spectrometry, and organic carbon was determined by Walkley and Black method.

The kencur rhizomes were dried in oven for 3 days and ground. The dried powdered kencur rhizome was extracted using maseration with methanol as solvent. The extract then evaporated to dryness. The bioactive compounds of kencur rhizome were determined using the gas chromatography-mass spectroscopy (GC-MS) method and analyzed at the DKI Jakarta Regional Health Laboratory, Central Jakarta, Indonesia. The GC-MS analysis was done using Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation Data System equipped with HP Ultra 2 Capillary Column (30 m in length x 0.20 mm in I.D x 0.11 $\mu$m in film thickness). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Helium gas was used as carrier gas with flow rate of 1.2 mL/min. The initial temperature at 80°C hold for 0 min, rising at $3^\circ\text{C}$/min to 150°C hold for 1 min, and finally rising 20°C/min to 280°C hold for 26 min. The bioactive compounds from different samples of kencur were identified based on GC retention time. The quantity of compounds was expressed as percentage based on peak area in the chromatogram.
3. Results and discussions

3.1. Kencur accession location characteristics
Location and climatic characteristics of *Kaempferia galanga* accession are exhibited in Table 1. Based on location, it is divided into two groups, including ≥ 600 m above sea level (highland) and < 600 m above sea level (lowland). The locations were chosen to represent six highland regions and six lowland regions. According to Bhuiyan *et al.* [6], kencur requires land with suitable agro-climate, which are the altitude ranges from 50 to 1200 meters above sea level (m asl), the annual average temperature ranges from 25 to 30°C, the number of wet months of 5-9 months per year, and dry months of 5-6 months. Table 1 also presents the climate data at the research location. Our results indicated that the temperature at the location ranges from 20 to 35°C. The lowest humidity of the location is 40%, whereas the highest humidity is 95%. The percentage of radiation intensity ranges from 60.9 to 75.5%. In general, the rainfall intensity of many locations is more than 1000 mm, and the lowest rainfall intensity is found in Madura.

**Table 1. Location and climate data of *Kaempferia galanga* accession survey**

| No. | Regency | Location       | Altitude (m asl) | T (°C)  | RH (%) | Light intensity (%) | Rainfall (mm³) |
|-----|---------|----------------|------------------|---------|--------|---------------------|----------------|
| 1   | Banyuwangi | Genteng       | 609              | 23 – 33 | 55 – 90 | 65.0                | 700 – 1.2000 |
| 2   | Blitar   | Wingi          | 129              | 21 – 33 | 45 – 90 | 69.0                | 1.000 – 1.5000 |
| 3   | Gresik   | Duduk Sampeyan | 15               | 25 – 35 | 40 – 85 | 72.2                | 1.000 – 1.5000 |
| 4   | Kediri   | Kandangan      | 89               | 21 – 33 | 60 – 95 | 64.4                | 1.000 – 1.5000 |
| 5   | Lumajang | Senduro        | 878              | 22 – 33 | 55 – 95 | 67.8                | 1.000 – 1.5000 |
| 6   | Madura   | Lenteng, Sumenep | 80           | 26 – 34 | 40 – 80 | 75.5                | 500 – 1.0000 |
| 7   | Mojokerto | Pacet          | 702              | 23 – 34 | 45 – 90 | 67.7                | 1.000 – 1.5000 |
| 8   | Malang   | Jabon, Tulungrejo | 718          | 20 – 32 | 55 – 95 | 60.9                | 1.500 – 2.0000 |
| 9   | Nganjuk  | Kebun Agung, Sawahan | 447    | 22 – 34 | 50 – 90 | 66.0                | 1.000 – 1.5000 |
| 10  | Pacitan  | Kledung, Bandar | 880             | 22 – 33 | 50 – 90 | 68.0                | 1.500 – 2.0000 |
| 11  | Ponorogo | Sooko          | 458              | 23 – 33 | 50 – 90 | 70.0                | 1.500 – 2.0000 |
| 12  | Trengalek | Puyung, Pule   | 995              | 20 – 33 | 55 – 90 | 68.0                | 1.500 – 2.0000 |
| 13  | Wonogiri | Miri, Kismartono | 351        | 23 – 34 | 50 – 85 | 72.9                | 1.500 – 2.0000 |

Table 2 shown the soil analysis of each location. All the kencur has grown in very dry soil, which has a water content of <15%. The soil in Banyuwangi had good characteristics. The soil samples from Banyuwangi contains the highest total nitrogen, available P₂O₅, cation exchange capacity (CEC), and organic carbon.
Table 2. Soil condition of Kaempferia galanga accession survey

| No. | Regency       | Water content (%) | Total N (%) | Available P2O5 (ppm) | CEC (me/100 g) | Organic C (%) |
|-----|---------------|-------------------|-------------|-----------------------|---------------|---------------|
| 1   | Banyuwangi    | 3.84              | 0.35        | 960                   | 4.74          | 4.29          |
| 2   | Blitar        | 2.00              | 0.06        | 158                   | 1.23          | 0.74          |
| 3   | Gresik        | 2.41              | 0.10        | 147                   | 1.46          | 1.05          |
| 4   | Kediri        | 4.98              | 0.05        | 39                    | 0.19          | 0.45          |
| 5   | Lumajang      | 4.29              | 0.17        | 194                   | 2.12          | 2.03          |
| 6   | Madura        | 2.84              | 0.15        | 32                    | 0.21          | 1.19          |
| 7   | Mojokerto     | 10.58             | 0.10        | 53                    | 1.12          | 0.82          |
| 8   | Malang        | 4.93              | 0.11        | 40                    | 2.22          | 0.96          |
| 9   | Nganjuk       | 3.71              | 0.05        | 42                    | 0.22          | 0.27          |
| 10  | Pacitan       | 6.02              | 0.21        | 41                    | 0.61          | 2.04          |
| 11  | Ponorogo      | 3.75              | 0.08        | 38                    | 0.52          | 0.77          |
| 12  | Trenggalek    | 4.07              | 0.18        | 247                   | 2.45          | 1.96          |
| 13  | Wonogiri      | 5.49              | 0.15        | 27                    | 0.66          | 1.41          |

Abbreviations: CEC - cation exchange capacity, N - nitrogen, C - carbon

3.2. Bioactive compounds present in Kaempferia galanga rhizome

The bioactive compounds present in each sample of kencur are shown in Table 3. The GC chromatograms of each sample are presented in Figures 2-3. Based on abundance, the top three major compounds present in the kencur rhizomes were ethyl p-methoxy cinnamate (EPMC) (61.46% - 79.8%), pentadecane (0.25% - 14.55%), and (Z)-ethyl cinnamate (1.47% - 10.01%). EPMC is an active constituent from kencur that is responsible for its medicinal properties, such as anti-inflammation and anti-angiogenesis [7]. The highest EPMC content of kencur was obtained from Malang (78.28%), which was lower than the sample from Wonogiri (79.8%) as a comparison. Based on EPMC content, it can be concluded that the regency with highest until the lowest EPMC are Malang (78.28%), Blitar (77.23%), Trenggalek (73.33%), Ponorogo (72.87%), Banyuwangi (71.72%), Gresik (71.64%), Kediri (71.63%), Pacitan (71.52%), Madura (71.29%), Lumajang (63.36%), Nganjuk (63.01%), and Mojokerto (61.46%).

Table 3. Bioactive compounds from Kaempferia galanga rhizome

| No. | Compounds | BWI (%) | BLT (%) | GRS (%) | KDR (%) | LMG (%) | MDR (%) | MLG (%) | MJK (%) | NGK (%) | PCT (%) | PNR (%) | TRG (%) | WNG (%) |
|-----|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1   | Pentadecane | 7.59    | 6.98    | 3.26    | 13.81   | 3.27    | 3.67    | 10.16   | 23.94   | 8.44    | 0.25    | 14.55   | 11.62   | 5.89    |
| 2   | Ethyl p-methoxy cinnamate | 71.72 | 77.23 | 71.64 | 71.63 | 63.36 | 71.29 | 78.28 | 61.46 | 63.01 | 71.52 | 72.87 | 73.33 | 79.8 |
| 3   | (Z)-Ethyl cinnamate | 5.77 | 1.56 | 7.69 | 71.14 | 13.17 | 3.56 | 6.91 | 6.26 | 10.01 | 1.47 | 5.46 | 7.55 |
| 4   | 8-heptadecene | 1.21 | 1.49 | 1.62 | 2.05 |
| 5   | Bicycle [7.1.0] dec-2-ene | 1.1 |
| 6   | 5-formyl-6-methyl-4,5-dihydropyran | 1.69 |
| 7   | (Z,Z)-3,9-cis-6,7-epoxy-nondecadiene | 1.69 |
| 8   | 4- tetradecyne | 1.06 |
| 9   | Cyperene | 1.33 |
| 10  | Ethyl (2e) -3- phenyl -2-propene | 1.25 |
| 11  | 8-isopropyl-1-methyl-5-methylene, 6-cyclodecadiene | 1.38 |
| 12  | Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- | 1.9 |
| 13  | 4-Methylene-tricyclo [6.2.1.0(2,7)] unduecane | 1.22 |
| 14  | (1r,2s,5s,5r,110s)-1,6-dimethyl-10-ethoxy-11-oxatricyclo [5.3.0.1 (2,5) ] -6-undecen-9-one | 1.22 |

Abbreviations: BWI - Banyuwangi, BLT - Blitar, GRS - Gresik, KDR - Kediri, LMG - Lumajang, MDR - Madura, MJK - Mojokerto, MLG - Malang, NGK - Nganjuk, PCT - Pacitan, PNG - Ponorogo, TGR – Trenggalek, WNG – Wonogiri.
Figure 2. Chromatogram of the bioactive compounds present in (a) Banyuwangi, (b) Blitar, (c) Gresik, (d) Kediri, (e) Lumajang, (f) Madura, (g) Malang, (h) Mojokerto, (i) Nganjuk, and (j) Pacitan.
Plants can have potential as a medicinal plant because they have secondary metabolites which are resulted from the adaptation process of plants to the environment or stress [8]. Plants produce secondary metabolites with various structures, functions, and contents. A study from Karimi et al. [9] revealed that geographic, climatic, and edaphic factor are linked to variation of secondary metabolite profile of plant. Sunlight affects the production of secondary metabolites because plants use sunlight for photosynthesis. Kencur plants will produce secondary metabolites under conditions of incomplete sunlight radiation intensity (shade-loving plant). It was showed in this study which is light radiation intensity ranged from 60.9-75.5%. The results of photosynthesis in the form of carbohydrates are then processed and will become bioactive compounds. Meanwhile, the increase in humidity will stimulate the formation of secondary metabolites as a physiological defense mechanism [10]. According to Srivastava et al. [11], in addition to environmental conditions, the chemical content of the soil, especially the content of potassium and phosphorus, significantly affects the bioactive content that will be formed in the kencur rhizome. The research of Maheswarappa et al. [12] showed that kencur plants planted with coconut shade affected the production of kencur plant rhizomes. The shaded kencur plants have a high percentage of nutrient content.

4. Conclusion
The bioactive compound in kencur rhizomes is influenced by the location. Based on the abundance, the top three major compounds present in the kencur rhizomes are ethyl p-methoxycinnamate (EPMC) (61.46% - 79.8%), pentadecane (0.25% - 14.55%), and (Z)-ethyl cinnamate (1.47% - 10.01%). Kencur samples from Malang, Blitar, and Trenggalek contain a higher EPMC content than other accession locations in East Java. Thus they can be the source of kencur rhizomes for further research.

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