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Research Article
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The Microelement Analysis, Physicochemical Properties of Ethyl Acetate Fraction of *Kaempferia galanga* L. and Its Lipid Peroxidation Inhibitory

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ABSTRACT: *A previous study reported that Kaempferia galanga* L. *(K. galanga) has anti-inflammatory and antioxidant activity. However, the research about how Kaempferia galanga* L. *inhibits lipid peroxidation (LPOI) has been limited. This study aimed to determine the physicochemical properties of the ethyl acetate fraction of K. galanga (EAFK) and its lipid peroxidation inhibitory activity. The anatomical structure of the rhizome was observed using a light microscope and scanning electron microscopy (SEM). Microelement analysis was determined by SEM-Energy Dispersive X-Ray Spectroscopy (SEM-EDX) mapping. The 70% ethanol extract of K. galanga was fractionated by a liquid-liquid technique using ethyl acetate. Phytochemical, the total phenolic and flavonoid levels, ethyl-p-methoxycinnamate (EPMC) as a marker compound, the specific and non-specific parameters of the fraction were determined. The antioxidant activity was evaluated using DPPH and LPOI assays. The results showed that the rhizome of K. galanga has oil cells, and the powder contains microelements. The EPMC level in the EAFK was 0.66 % (w/w). At concentrations of 4000 ppm and 625 ppm, the EAFK could inhibit 15.90%±0.35 of DPPH free radical scavenging and 17.43%±6.52 of lipid peroxidation, respectively. Based on these findings, K. galanga contains microelements, EPMC, and the EAFK has an antioxidant effect via inhibiting lipid peroxidation and antiradical activity.*

Keyword: *antioxidant; Kaempferia galanga; lipid peroxidation inhibitory; microelement; SEM-EDX*

1. Introduction

Kaempferia galanga L. is a rhizome from the Zingiberaceae family and locally known as kencur in Indonesia. It has a strong aroma and was used as a spice and herbal medicine. The *K. galanga* is native to India and spreads in Myanmar, China, Malaysia, Thailand, and Indonesia [1]. The specific aroma of *K. galanga* and its use as a herbal medicine are related to its anatomical structure and chemical compounds. In Indonesia, especially among the Javanese, *K. galanga* is used to cure a cough and skin inflammation. This plant is consumed as "jamu beras kencur" as a herbal drink. The rhizome of *K. galanga* is grated and mixed with rice flour, then boiled. Hikmawanti et al reported that 70% ethanolic extract of *K. galanga* contained ethyl-trans-p-methoxycinnamate (EPMC), ethyl cinnamate trans, methyl 4-methoxycinnamate, ethyl-cis-p-methoxycinnamate, benz[A]azulene-1,4-dione, and 2,3-dimethyl- [2].

K. galanga is plentiful in essential oil. Wang et al. (2023) stated that *K. galanga* essential oil (KGEO) could inhibit lipid peroxidation by increasing superoxide dismutase (SOD) and decreasing the formation of malondialdehyde (MDA) in induced H_2O_2 Zebrafish [3]. The oxidation of polyunsaturated fatty acids (PUFAs) leads to the formation of lipid peroxides (ROOH) and is present in every tissue and body fluid. Lipid peroxide can be converted to MDA by metal ions. Oxidative stress can cause lipid peroxidation, and leading to cell death. Antioxidants can detain or inhibit lipid peroxidation or other molecules by inhibiting or eliminating the initiation or propagation of excess reactive species.

KGEO, which is distilled from *K. galanga* rhizome in Bali, reported high antioxidant activity in DPPH free radical-scavenging assay with an IC_{50} value of 85.24 to 89.19 µg/mL [4]. Dwita et al. (2021) stated that the ethyl acetate fraction of *K. galanga* was able to inhibit oxidative stress better than the water fraction and n-hexane fraction through observation of MDA concentration in rats experiencing inflammation induced by carrageenan [5]. However, observation of ex vivo inhibition of lipid peroxidation (LPOI) in the ethyl acetate fraction of *K. galanga* has never been reported.

Herbal materials should be authenticated to a quality that complies with the standards outlined in the National Pharmacopeia. The study aimed to evaluate the inhibition of LPOI in homogenates of normal rat liver, and to figure out the microscopic description of the rhizome, analyze the microelements of *K. galanga* powder (analysis using SEM-EDX mapping. EDX mapping shows the spatial distribution of elements in the scanned area; a different color represents each element), and the specific and non-specific characteristics of ethyl acetate fractions of *K. galanga*.

2. Materials dan method

2.1. K. galanga plant

The rhizome of *K. galanga* was derived from the Kebun Biofarmaka, IPB University, Bogor, West Java, Indonesia. Its voucher code is BMK0260102016.

2.2. Microscopic observation

The fresh rhizome of *K. galanga* was sliced as thinly as possible and mounted on a slide. A small amount of chloral hydrate was added to the slice, then covered with a cover glass and examined under a light microscope (Olympus®) at 400x magnification. The rhizome was also dried and powdered for morphological examination using a Hitachi® SU3500 scanning electron microscopy (SEM). Additionally, microelement analysis for Fe, Cu, Zn, and Se was performed using SEM coupled with energy dispersive X-ray (SEM-EDX).

2.3. Extraction and fractionation

A total of 5 kg of *K. galanga* powder was extracted by maceration using 70% ethanol as

the solvent at a sample-to-solvent ratio of 1:5 (b/v). The maceration process was conducted over three consecutive 24-hour periods. After each 24 hours, the filtrate was collected, and fresh 70% ethanol was added in the same ratio for the subsequent extraction cycle. The filtrate was concentrated using a rotary evaporator at 50 °C until the ethanol content was reduced to below 5%. Subsequently, 500 mL of *K. galanga* extract, with a viscosity of 1.1%, was dried in a glass dish at 50 °C for two days.

Additionally, 2.5 L of *K. galanga* extract (equivalent to 220 g) was subjected to liquid-liquid partitioning using n-hexane and ethyl acetate [5]. The ethyl acetate fraction was concentrated under vacuum using a rotary evaporator until a thick consistency was obtained. Furthermore, this fraction is called the ethyl acetate fraction of *K. galanga* (EAFK). Organoleptically, EAFK is observed for its shape, smell, taste, and color. The yield of EAFK (in percent, %) was calculated against the weight of the fractionated ethanol extract (g).

2.4. Evaluation of EAFK-specific parameters

2.4.1. Fluorescence analysis of the EAFK

Fluorescence analysis of the EAFK was treated using various reagents, such as 2 N HCl, 2 N H_2SO_4, 4 N HNO_3, and 1 N NaOH in a drip plate. Then, the fluorescence of each mixture was examined under visible light and ultraviolet (UV) light at 254 and 365 nm using a UV box (CAMAG®, Germany) [6].

2.4.2. Water-soluble and ethanol-soluble analysis of the EAFK

Water-soluble and ethanol-soluble analysis of the EAFK was macerated using chloroform-saturated water (for water soluble analysis) and 95% ethanol (for ethanol soluble analysis), separately, in a ratio of sample-solvent at 5:100 for 24 h. The filtrate was filtered and evaporated at 105^0C, until dry and reached a constant weight. The water and ethanol soluble content (in percent, %) were calculated against the EAFK weight [7].

2.4.3. Phytochemical analysis of the EAFK

Qualitative phytochemical analysis of EAFK was conducted to detect the presence of flavonoids, phenols, tannins, alkaloids, saponins, and terpenoids in the ethyl acetate fraction. The procedure was performed according to the following methods: The Indonesian Herbal Pharmacopoeia standards [7].

Total phenol level was measured following Hikmawanti et al. (2024) [8]. The EAFK solution was prepared in methanol. Gallic acid as a standard was performed in serial concentrations at 12.5, 25, 50, 100, and 200 µg/mL. Briefly, 20 µL of sample (gallic acid or EAFK) was mixed with 100 µL of Folin-Ciocalteu solution (1:10). After 4 min, 75 µL of 7,5% Na_2CO_3 solution was added to the mixture, which was then incubated at room temperature for 2 hours in the dark. The optical density was measured at 750 nm using a Bio-Rad® microplate reader, with methanol used as the blank. The assay was performed in triplicate.

The total flavonoid content of EAFK was determined according to the method described by Sembiring et al (2018). The EAFK solution was prepared in methanol with quercetin used as a standard. Serial concentrations of quercetin in methanol were prepared at 12.5, 25, 50, 100, and 200 µg/mL. Briefly, 50 µl of either the sample or standard were placed into each well, followed by the addition of 10 µl of 10% aluminum chloride, 10 µl of 1M sodium acetate, and 150 µl of methanol. The mixture was incubated at room temperature for 40 minutes. Absorbance readings were measured at 415 nm using a Bio-Rad® microplate reader, with methanol used as a blank. All assays were performed in triplicate [9].

2.4.4. Identification of EPMC of the EAFK

Identification of EPMC was carried out using thin layer chromatography (TLC) combined with densitometry. Ethyl acetate fraction (1%) and ethyl-p-methoxycinnamate (0.1%, used as a standard) were prepared in ethanol and analyzed in triplicate. Both solutions were applied 2 µL/spot onto a silica gel 60 F_{254} (Merck®) plate, which

had been pre-activated by heating in an oven at 110 °C for 60 minutes. The plate was developed using a mobile phase of toluene and ethyl acetate (95:5, v/v). The appearing spots were visualized under UV light at 254 nm. The Rf values were calculated by dividing the distance traveled by each component by the distance traveled by the solvent [7].

2.5. Evaluation of EAFK non-specific parameters

Non-specific parameters observed from EAFK include total ash, moisture content, loss on drying, heavy metal content (Pd and Cd, using atomic absorption spectrophotometer), and microbial contamination (total plate count, coliform, and mold/yeast). The test was conducted at the Biopharmaceutical Laboratory (ISO 17025), IPB University, Bogor, West Java. The procedure follows the Indonesian Herbal Pharmacopoeia.

2.6. Antioxidant property testing

2.6.1. DPPH Assay

Several sample solutions of EAFK were prepared with concentrations of 1000, 2000, and 4000 ppm and quercetin with 62.5, 125, and 250 ppm concentrations. The 20 µL sample was pipetted and 180 µL DPPH solution (0.1 mM) was added onto a 96-well clear polystyrene microplate and then shaken for 60 seconds. The mixture was incubated at room temperature for 30 minutes. DPPH exhibits a purple colour in methanol and fades to shades of yellow when antioxidants are present. The absorbance was measured with a microplate reader at a length of 595 nm. Quercetin was used as a positive control, subjected to the same treatment as the sample. The percentage of DPPH scavenging was determined using Equation 1:

$$\% \text{ DPPH scavenging} = \frac{A_0 - A_1}{A_0} \times 100\% \quad \text{..... (1)}$$

A_0 represents the absorbance of the control, while A1 denotes the absorbance of the extracts or standard. The experiment was conducted in triplicate for each concentration [10].

2.6.2. Lipid peroxidation inhibition (LPOI) assay

The method of LPOI was carried out based on Kumar et al. (2013) [11]. The 10% liver rat homogenate method was used refers to Hikmawanti et al. (2025) [12]. The homogenate was then centrifuged at 12,000 rpm for 15 minutes at 4°C, and the resulting supernatant was used for the in vitro lipid peroxidation assay. For the assay, 0.5 mL of the supernatant was mixed with 1 mL of 0.15 M KCl and 0.3 mL of extract or standard at varying concentrations (1000, 2000, 4000 ppm).

Lipid peroxidation was triggered by the addition of 300 µL of 0.5 mM $FeCl_3$ to the reaction mixture, which was incubated at 37 °C for 30 minutes. The reaction was halted by adding 2 mL of ice-cold Thiobarbituric acid (TBA)-Trichloroacetic acid (TCA)-HCl-BHT solution. The supernatants were collected, and the absorbance of the resulting pink-coloured complex was measured at 532 nm using a spectrophotometer. The extent of lipid peroxidation was assessed by determining the levels of Thiobarbituric acid reactive substance (TBARs). A control assay was performed using PBS in place of the extract. The percentage of lipid peroxidation inhibition in the samples was calculated according to Equation 2:

$$\% \text{ LPOI} = \frac{A_0 - A_1}{A_0} \times 100\% \quad \text{.....(2)}$$

A_0 represents the absorbance of the control (containing 300 µL of distilled water), and A1 corresponds to the absorbance of the sample with extract or standard. The percentage of inhibition was then plotted against concentration, and data were analyzed from the resulting graph. All experiments were conducted in triplicate and repeated three times for each concentration.

3. Result and disscussion

3.1. Microscopic observation

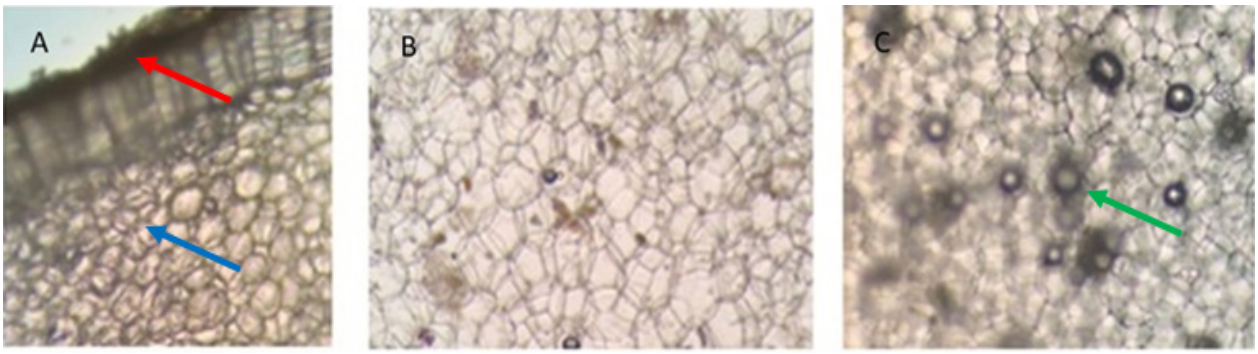

Figure 1. Cross-section of *K. galanga* rhizome at 40 × 10 magnifications. Notes: epidermis (red arrow) and cortex (blue arrow) (A); parenchyma (B); magnification and oil cell (green arrow) (C).

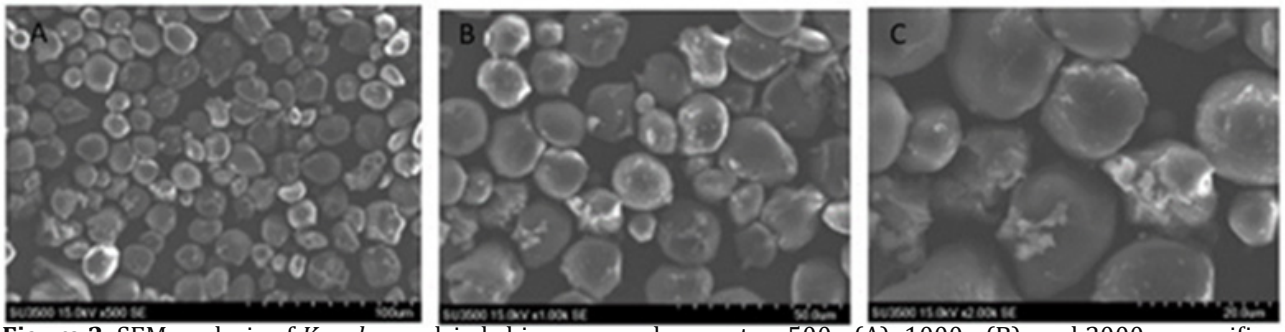

Figure 2. SEM analysis of *K. galanga* dried rhizome powder—notes: 500× (A); 1000× (B); and 2000× magnification (C).

Based on Figure 1, the rhizome of *K. galanga* features a layered and dense epidermal tissue, a broad parenchymal cortex, and contains oil cells or idioblasts. The idioblast cells appear yellowish and differ structurally from the surrounding tissues. Figure 2 presents a microscopic view of *K. galanga* powder captured by SEM at over 2000x magnification.

K. galanga is a rhizome belonging to the Zingiberaceae family, recognized for its distinctive aroma. This unique scent arises from essential oils stored within oil cells or idioblasts in the rhizome. Essential oils are secondary metabolites produced by plants and stored in idioblasts, which differ structurally and in content from the surrounding tissues. Figure 1 illustrates the abundance of oil cells within the parenchyma tissue of *K. galanga*. These oil cells are yellowish and visibly distinct from the surrounding cells, and can be observed under a light microscope at 100x magnification. The essential oil of *K. galanga* ranges in colour from yellow to pale yellow [13].

K. galanga primary constituent is ethyl-trans-p-methoxycinnamate (EPMC), along with other components such as 2-propenoic acid, caryophyllene, 3-carene, -muurolene, patchouli alcohol, and 1,8-cineole [13]. According to Raina et al. (2016), the essential oil of *K. galanga* rhizome contains 38 compounds, with the two major constituents being trans-ethyl-p-methoxycinnamate (28.4–70.0%) and trans-ethyl cinnamate (11.5–26.6%) [14]. Other notable compounds include 3-carene (0.1–6.5%), 1,8-cineole (0.2–5.2%), borneol (1.0–2.4%), pentadecane (6.0–16.5%). EPMC is the most abundant compound in *K. galanga* rhizomes, contributing to their aroma and flavour, and it exhibits various pharmacological properties [15].

Phenolic and flavonoid compounds present in *K. galanga* (Table 1), have been reported to possess various pharmacological activities, particularly antioxidant and anti-inflammatory effects. These bioactive compounds exert their activity through free radical scavenging mechanisms and modulation of inflammatory mediators [1].

Subaryanti et al. (2021) reported that idioblast cell density is higher in the pith tissue than in the cortical tissue [13]. Several factors influ-

Table 1. The specific parameters of EAFK

Parameters		Result
Organoleptics		Color: dark brown Shape: thick Scent: specific Flavor: pungent
Yield percentage		3.06% (w/w)
Phytochemical screening		Flavonoids, phenols, alkaloids, tannins, saponins, and terpenoids
Fluorescence analysis		
+ HCl	Visible Light	Light brown
	UV 254 nm	No fluorescence
	UV 365 nm	Dark green
+ H_2SO_4	Visible Light	Dark brown
	UV 254 nm	No fluorescence
	UV 365 nm	No fluorescence
+ HNO_3	Visible Light	Light yellow
	UV 254 nm	Bright yellow
	UV 365 nm	Yellow
+ NaOH	Visible Light	Bright yellow
	UV 254 nm	No fluorescence
	UV 365 nm	Greenish yellow
Water-soluble substances		62.11 % (w/w)
Ethanol-soluble substances		27.34% (w/w
Total phenolic compounds		235.591 mg GAE/g
Total flavonoid compounds		38.344 mgQE/g
EPMC level (using TLC-densitometry assay)		0.66% (w/w)

ence the density and composition of essential oils in the rhizome, including the rhizome maturity level, growth period, environmental conditions, post-harvest handling, size, and extraction methods [16,17]. These factors collectively impact the quantity and composition of essential oils produced [7]. Microscopic examination of *K. galanga* powder using SEM is shown in Figure 2. SEM images help to describe the physical appearance of the herbal powder particles, such as particle shape, surface texture, and agglomeration. Figure 2 reveals that *K. galanga* powder spherical particles are approximately 200 µm in size, visualised at 500x, 1000x, and 2000x magnifications at 15 kV. The surface structure often relates to extraction efficiency, adsorption, drying, or grinding processes.

SEM micrographs demonstrated that the powder possessed a spherical, some irregular, smooth compact surface, and some porous surface characteristics, suggesting mechanical disruption of the lignocellulosic plant matrix during grinding. Such morphological changes may enhance solvent accessibility and affect extraction efficiency, adsorption properties, and dissolution behaviour due to increased surface area and structural deconstruction [18].

Furthermore, microelement analysis of plant parts was conducted using the SEM-EDX mapping to determine the spatial distribution of microelements such as Cu, Fe, Se, and Zn in *K. galanga* powder. SEM-EDX mapping refers to analyzing combined results from SEM (Scanning Electron Microscopy) and EDX/EDS mapping (Energy Dispersive X-ray Spectroscopy mapping) to understand the surface morphology and elemental distribution of a sample. EDX mapping shows the spatial distribution of elements in the scanned area. Each element is represented by a different colour, such as blue spots for Cu, green spots for Fe, yellow spots for Se, etc.

EDX mapping demonstrated a uniform distribution of elements on the sample surface, indicating successful homogeneous synthesis of the composite. EDX analysis demonstrated the dominant elements, confirming the organic composition of the herbal powder. Minor elements were also detected, which may be associated with the

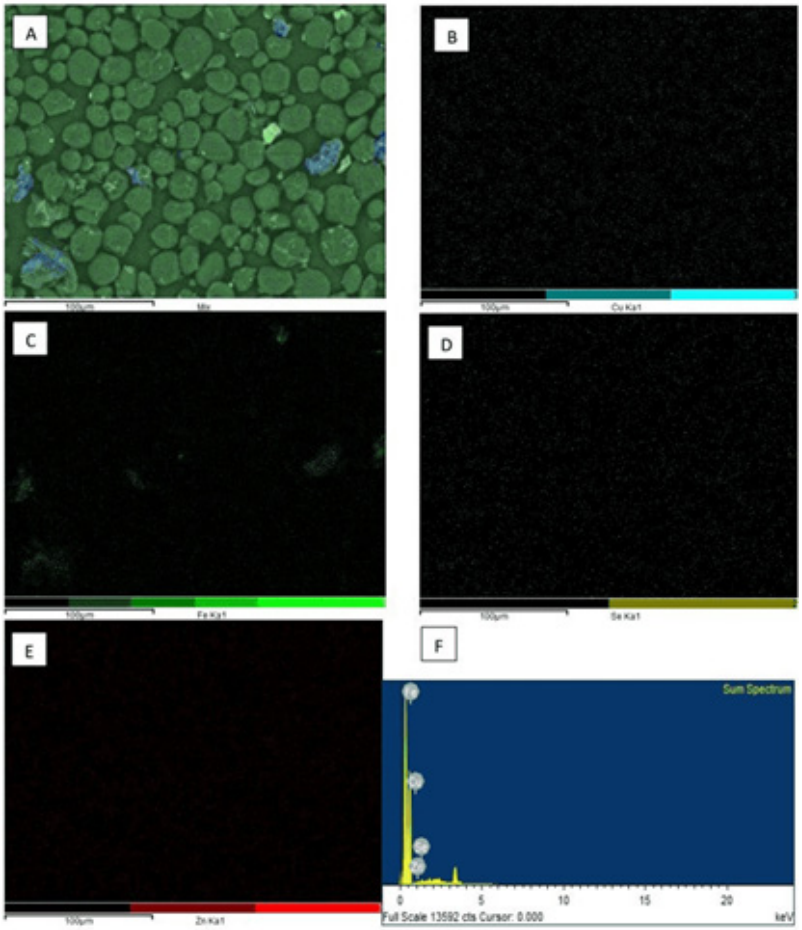

Figure 3. SEM-EDX microelement mapping analysis of *K. galanga* powder. Notes: mapping of Cu, Fe, Zn, Se (there were some color spots distributed accros the surface) (A); Cu mapping (there were blue spots distributed across the surface) (B); Fe mapping (there were green spots distributed across the surface) (C); Se mapping (there were yellow spots distributed across the surface) (D); Zn mapping (there were red spots distributed across the surface) (E); spectrum of microelements (F).

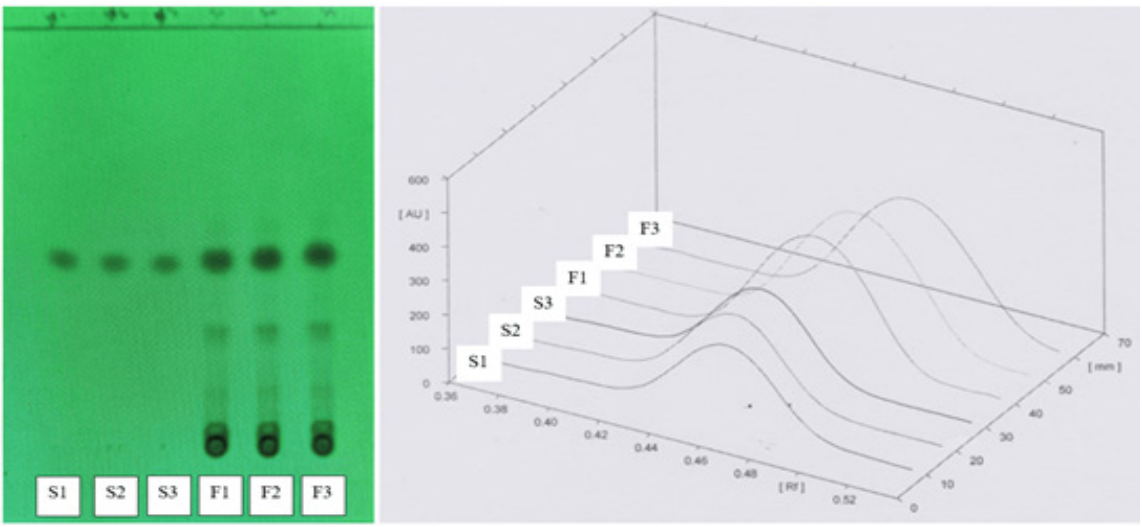

Figure 4. TLC chromatogram (left) and 2D-densitogram (right) of standard and EAFK scanned at 254 nm. Notes: S1, S2, S3 = standard EPMC at concentration of 0.1% (triple); F1, F2, F3 = EAFK at concentration of 1% (triple); mobile phase = toluene: ethyl acetate (95:5); stationary phase = TLC aluminium plate silica gel $GF_{254.}$

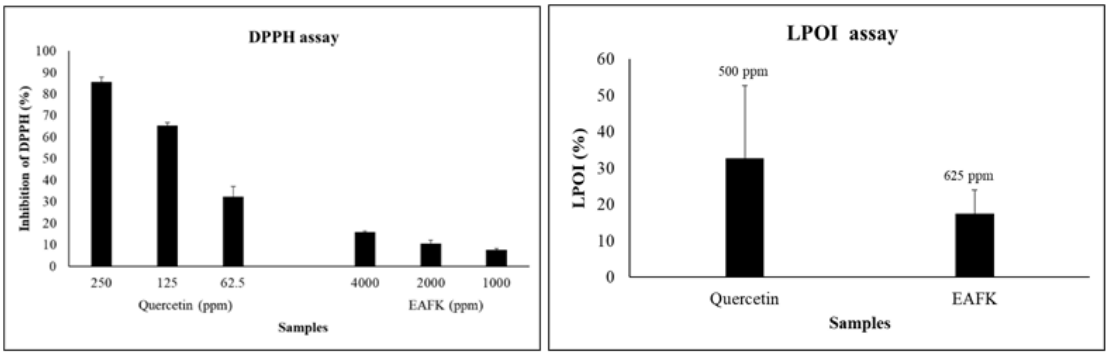

Figure 5. Free radical scavenging activity of EAFK. DPPH assay (a) and LPOI assay of EAFK (b) used quercetin as a positive reference. Note: EAFK = ethyl acetate of *K. galanga.*

natural mineral content of the plant material [19]. In SEM-EDX, a sum spectrum (or summed spectrum) refers to the cumulative EDX signal obtained from a selected analysis area or from multiple scanned regions during elemental mapping. This spectrum represents the overall elemental composition of the analyzed region rather than the composition at a single spot, thereby providing an average elemental distribution of the sample surface. The spectrum can show dominant peaks, indicating that the sample mainly consisted of its compounds. The results of the SEM-EDX mapping analysis are presented in Figure 3.

Macroelements and microelements present in plants play significant roles in their function as nutrients or medicinal agents for disease prevention [20]. The macroelements in plants include elements such as carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). Meanwhile, microelements or also known as trace elements, encompass non-toxic elements like chromium (Cr), sodium (Na), magnesium (Mg), potassium (K), cobalt (Co), iron (Fe), calcium (Ca), vanadium (V), manganese (Mn), nickel (Ni), zinc (Zn), selenium (Se), and copper (Cu). However, plants may also contain toxic elements such as arsenic (As) and lead (Pb). Microelements in plants contribute to various physiological processes and act as cofactors in enzyme activity [21]. Both the excess and deficiency of microelements can influence plant toxicity [22]. Iron (Fe) in particular, is an essential microelement in living organisms. It plays a crucial role in numerous physiological functions, including oxygen transport, DNA synthesis, and cellular energy production [23]. Iron deficiency can lead to various health issues, such as anemia, chronic fatigue, stunted growth, and inflammation [24,25]. SEM-EDX analysis indicates that *K. galanga* powder contains Iron (Fe) and has a peak spectrum from SEM-EDX analysis (Figure 3 (F)), suggesting its potential benefit in helping to prevent iron deficiency and support metabolic processes in the body.

Copper (Cu) is an essential element that enables the proper function of several enzymes for metabolism. It is an important component in connective tissue, bones, the brain, the liver, and other organs [26]. A copper deficiency can lead to liver damage, malabsorption issues, and depigmentation of hair and skin. *K. galanga* powder contains copper, which may help contribute to the body copper intake. Zinc (Zn) is an important microelement, as it influences the expression and activity of transcription factors. In silico studies have shown that approximately 10% of the body's proteins can bind to zinc. Zinc deficiency can result in growth impairment, as well as disorders of the nervous and endocrine systems, and it compromises the immune system in mammals [27]. Zinc obtained from food or breast milk is absorbed in the body via several zinc transporters in the intestine.

Selenium (Se) is a microelement that plays several crucial roles in the body, including as an antioxidant, preventing carcinogenesis, and reducing inflammation [28], aiding in detoxifica-

Table 2. The non-specific parameters of EAFK

Parameters	Results
Total ash	2.47 % (w/w)
Moisture content	1.19 % (w/w)
Loss on drying	7.64% (w/w)
Heavy metals levels	
Cd	Not detected
Pb	Not detected
Microbial contamination	
Total plate count (TPC)	Negative
Coliform test	Negative
Mold/yeast test	Negative

tion, modulating the immune system, preventing carcinogenesis, supporting thyroid function, and contributing to sperm maturation and motility [1]. A selenium deficiency can lead to infertility and disorders of the nervous and immune systems. Based on the spectrum analysis of *K. galanga* powder, the elemental composition includes Fe (-11.65%), Cu (11.88%), Zn (21.15%), and Se (78.62%). These findings indicate that *K. galanga* contains microelements that are significant for various physiological processes in the human body.

3.2. EAFK specific and non-specific parameters

The chemical constituents of EAFK are shown in the phytochemical analysis results presented in Table 1. These findings reveal that EAFK contains flavonoids, phenols, alkaloids, tannins, saponins, and terpenoids. Table 2 showed that EAFK has no metal contamination, such as Pb and Cd, and is negative for microbial contamination, such as coliform and mold/yeast. These results demonstrated that the EAFK has good standard quality. *K. galanga* contains EPMC (0.66%, w/w) using TLC densitometry as shown in Figure 4.

This study found that all samples showed antioxidant activity compared to quercetin. Figure 5 shows the free radical scavenging activity of the EAFK. Among the samples tested, the ethyl acetate fraction at a concentration of 4000 ppm exhibited the highest DPPH free radical scavenging, reaching 15.90%±0.35.

Based on ethnomedicine data by Wang et al. (2021), *K. galanga* is widely used as a treatment in Asian regions such as China, Malaysia, India, Thailand, and Indonesia. *K. galanga* has antioxidant activity. Most of compound contained in *K. galanga* is EPMC. Several studies have demonstrated that EPMC exhibits anti-inflammatory properties by lowering the levels of leukotriene B, a pro-inflammatory protein, inhibiting protein kinases involved in cell activation signaling during inflammation, and showing strong antioxidant activity through the DPPH method [7,29]. EPMC can protect from oxidative cellular damage [30]. The underlying mechanism of the DPPH method is that it involves hydrogen atoms of antioxidant compounds bonding with free electrons in compounds radical causing a change of free radicals (diphenyl picrylhydrazyl) to non-radical compounds (diphenyl picrylhydrazine). This is indicated by a change in colour from purple to yellow (free radical compounds reduction in the presence of antioxidants) [11].

Figure 5 showed that the lipid peroxidation inhibition activity of the ethyl acetate fraction of *K. galanga* at a concentration of 625 ppm had higher inhibitory activity (17.43%±6.52) compared to other concentrations and nearly equivalent to quercetin. Lipid peroxidation is generally defined as the process in which oxidants, such as free radicals, target lipids with carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs). This research used the TBARs assay to measure the LPOI.

Lipid peroxidation was induced in rat liver homogenate using iron, leading to the formation of lipid peroxidation products like malondialdehyde

(MDA) or other degradation products that react with TBA to form a pink chromogen whose absorbance is measured with a UV-Vis spectrophotometer at 532 nm. The reaction rate between MDA and TBA depends on temperature, pH, and TBA concentration. The maximum pigment intensity is obtained in 60 minutes of heating, and the reaction rate is faster in acidic conditions. Lipid peroxidation can result in cell damage or cell death. Inhibition of lipid peroxidation is regarded as a key indicator of antioxidant activity [9].

The findings suggest that *K. galanga* can prevent cellular damage induced by free radicals by interrupting the chain reactions that drive lipid peroxidation. Therefore, *K. galanga* represents a valuable source of natural antioxidants and holds potential for treating various diseases associated with free radical damage. Hussain et al (2021) showed that treatment with *Cannabis sativa* and *Morus nigra* in iron-induced goat liver to concentration-dependent inhibition of the TBARS production, bringing the levels close to the basal values [31]. Their findings demonstrated that the plant extracts suppressed TBARs formation induced by Fe (II) and SNP in goat liver homogenate across various concentrations. The antioxidant effect of these plant extracts is likely do to their bioactive compounds, which warrant further investigation in upcoming research.

4. Conclusion

The rhizome of *K. galanga* contains spherical cells approximately 200 µm in size and idioblast cells rich in essential oils. It also comprises microelements such as Fe, Cu, Zn, and Se, along with secondary metabolites, including flavonoids, phenols, alkaloids, saponins, tannins, and terpenoids. The EPMC level in the ethyl acetate fraction of *K. galanga* (EAFK) was 0.66 % (w/w). At concentrations of 4000 ppm and 625 ppm, the EAFK could inhibit 15.90%±0.35 of DPPH free radical scavenging and 17.43%±6.52 of lipid peroxidation, respectively. Based on these findings, the ethyl acetate fraction of *K. galanga* reaches quality standards and has antioxidant activity. This fraction is potentially developed as a raw material for herbal medicine.

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