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Ultrasonic–assisted Extraction of *Kaempferia galanga* Essential Oil and Its Antioxidant Activity

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Abstract
Kaempferia galanga is one of the plant species that has been used as a medicinal herb that usually located in India and Indochina. This species belongs to the Zingiberaceae family, which is commonly called the ginger family. This species is an enormous potential market for the essential oil business because of its beneficial properties as a treatment to treat metabolic disorders, inflammation, urinary tract infections, fevers, coughs, hypertension, and a variety of other conditions. Studies on *K. galanga* have shown that it contains a variety of chemical constituents in the essential oil and possesses good antioxidant properties. This study aims to determine the chemical constituents in the rhizome and leaf of *K. galanga*. Apart from that, determination of the total phenolic content (TPC) of *K. galanga* essential oil and antioxidant activity of *K. galanga* were also performed in this study. Essential oil from the leaves and rhizomes of *K. galanga* was extracted by using a hydrodistillation process assisted by pretreatment of ultrasonic extraction (UAE) with the temperature (40°C), frequency (50 Hz) and time (30 minutes). Determination of chemical constituent of *K. galanga* was performed by using Gas Chromatography (GC) and Gas Chromatography Mass Spectroscopy (GC-MS). The primary constituents identified were ethyl p-methoxycinnamate (62.55%), pentadecane (15.56%) and ethyl cinnamate (15.54%). TPC was performed with Follin – Ciocalteu colorimeric using gallic acid as a standard and measured at 760 nm. The results obtained for TPC were 8.962, 2.391, 10.589 and 2.438 mg GAE/g for oil extraction from rhizomes and leaves. The antioxidant activity was determined by 2,2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and measured at 517 nm. A serial dilution at 1000, 500, 250, 125, 62.5, and 31.25 ppm was used in this assay. The essential oil extracted from the rhizome with pretreatment of UAE showed the best antioxidant properties as it exhibited radical scavenging activity towards DPPH with an IC50 value of 30.66 mg/ml. Based on the findings, *K. galanga* can be considered as an excellent natural resource for pharmaceuticals and a reliable source of natural oxidant

Keywords: *Kaempferia galanga*, essential oil, ultrasonic-assissted, antioxidant activity, total phenolic content

1. Introduction

K. galanga is one of the natural products specifically in plants that has been used as a medicinal herb around the world [1]. *K. galanga,* commonly known as kencur or cekur, *K. galanga* can usually be found in India and Indo-China. The extraction methods that can be used include hydrodistillation, steam distillation and organic solvents extractions. Extraction of essential oil with the pretreatment of ultrasonic-assisted extraction involves ultrasound waves [2]. Ultrasound-assisted extraction (UAE) has been effectively utilized to extract a variety of chemical compounds from plants, including phenolic compounds, flavonoids, polysaccharides, alkaloids, and glycosides. UAE has become more appealing than traditional extraction procedures due to a variety of benefits, including higher extraction yields [3].

Plant species still need to be researched to discover their specialities, which could help the study of medicine and pharmacology in the future. Because natural products are organic chemical compounds that need extraction for different spectroscopy purposes, extraction through hydrodistillation and

ultrasonic-assisted extraction is implemented to extract the essential oil. Thus, for this study, *K. galanga*, a medicinal herb plant, is chosen for the research of essential oil extraction and chemical constituents. Moreover, the effect of ultrasonic-assisted extraction pretreatment was examined during the extraction processes. In addition, identification of the chemical constituents and their antioxidant activity, by gas chromatography- mass spectroscopy (GCMS) and free radical scavenging by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was conducted.

2. Literature Review

2.1. Species of Kaempferia galanga and its traditional application

K. galanga, commonly known as ginger, is a medicinal herb used for decades in Malay traditional medicine and Traditional Chinese Herb Medicine [4]. *K. galanga* is widely used in today's world of medication, where a large population of people consume this plant for its benefit in health [5]. Other than that, *K. galanga* is also commonly used as a spice in culinary, especially in Malaysia as nasi ulam. Furthermore, because *K. galanga* has a strong common feature of balmy scent, it become as one of the main component in a odurizer, which is claimed to improve sleep or reduce stress in Japanese people [6]. *K. galanga* is popular to treat metabolic disorders, inflammation, urinary tract infections, fevers, coughs and hypertension.

2.2. Ultrasonic assisted extraction (UAE)

UAE is a modification method of extraction by applying a high frequency of sound and a small amount of solvent in the sample to produce an effective extraction of compounds [7]. UAE is used because it is an expeditious and effectual extraction technique that uses ultrasound to create rapid solvent movement, resulting in a mass transfer in a high speed and have extraction acceleration [1]. The advantage of using the ultrasonic extraction method for this study is that ultrasound-assisted extraction has grown more appealing compared to traditional extraction procedures due to numerous advantages, including less energy consumption, shorter extraction times, less active component destruction, and higher extraction yields [8].

2.3. Chemical constituent of K. galanga by GC and GCMS

Gas chromatography (GC) is one of the chromatography used in analytical chemistry, also popular to be used in organic and inorganic chemistry to separate and analyse compounds. The chemical constituent of *K galanga* through its essential oil are ethyl-p-methoxycinnamate and trans-ethyl cinnamate as the major compound identified, including methyl cinnamate [9]. These chemical compounds are extremely important for antioxidant activity, antimicrobial properties, and much more. Therefore, *K. galanga* is a highly valued medicinal plant that has a wide range of pharmacological applications.

2.4. Total phenolic content (TPC)

Total phenolic content (TPC) is an activity to determine the specific amount of phenolic contents in the compound that has been analysed [10]. This method is used for the determination of phenolic and polyphenolic antioxidants. Phenolic compounds in the plants have redox properties, allowing them to function as antioxidants. The Folin-Ciocalteu method is a reducing capacity and phenolic concentration test based on electron transfer [11]. There are many different types of phenolic compounds, ranging from simple groups to complex groups that can bind to glucose groups as glycols. The total phenolic content of the sample is determined to measure antioxidant activity in the plant is evaluated [10].

2.5. Antioxidant activity of genus of Kaempferia by 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activities of *K. parviflora* rhizomes were measured using DPPH free radical scavenging activity and showed significant differences. Ascorbic acid was used as a standard.The decolouration of the initial color is proportional to the test substances having anti-radicalizing power. Since the lower the IC50 values, the higher the antioxidant activity of the sample have, possessed the highest radical scavenging property.

3. Methodology

3.1. Plant material

Fresh leaves and rhizomes of *K. galanga* were purchased from local market Pontian, Johor. The leaves and rhizomes of *K. galanga* were cut into the smallest pieces. Once all the fresh sample has been cut into small pieces, it was used for hydrodistillation activity to obtain the essential oil.

3.2. Pretreatment by Ultrasonic-assisted extraction

Distilled water was used as a solvent in the extraction process. A sonicator was used to generate the ultrasound effect. The ultrasound equipment (Elmasonic S 70 H, D-78224 Singen – Gottlieb – Daimler-Str.17, Elma Schmidbauer GmbH, Germany) is used for the pretreatment. The ultrasonic device could induce cavitation with a bubble implosion effect to help extraction. The parameters were controlled using a water bath placed below the extraction setup the temperature (40°C), frequency (50 Hz) and time (30 minutes).

3.3. Extraction of essential oil by hydrodistillation

A fresh sample transferred into a round bottom flask, filled with distilled water with a ratio (1:5) and mixed well. The Clevenger apparatus was set up for hydrodistillation purposes in which the knob must be tightly closed to prevent any leakage. The essential oil sample was collected after 8 hours of hydrodistillation process. The knob was loosed slowly and the essential oil was collected in a vial. Distillate from the Dean-Stark apparatus was extracted using 10 mL diethyl ether (Et_2O) in a separatory funnel, and subsequently dried over anhydrous magnesium sulphate ($MgSO_4$) for 10 minutes. The $MgSO_4$ was filtered, and the filtrate was allowed to dry for several hours to evaporate all the Et_2O.

3.4. Identification of chemical constituents of Constituents by Gas Chromatography (GC) and Gas Chromatography – Mass Spectroscopy (GC-MS)

The identification of chemical constituents in the essential oil sample was obtained by quantitatively analysing the composition of the essential oil (GC-MS) and GC Hewlett-Packard gas chromatography, which is equipped with semi- polar capillary column with a dimension of 25 m × 0.25 µm × 0.25 µm. Helium gas (He) was used as a carrier gas (mobile phase) and set as temperature programming. The operating conditions were as follows: injection temperature: 250°C, initial oven temperature: 50°C, final oven temperature: 250°C, ramp rate: 5°C min –1, a flow rate of He carrier gas: 1.0 mL min–1, electron impact energy: 70 eV. The spectra obtained was compared with the mass spectral data that has been equipped with the GC-MS and GC instruments [15].

3.5. 2,2-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activity of *K. galanga* leaves and rhizomes was determined using DPPH [12] with some modifications. DPPH solution was made by using reagent (80 g/mL), dissolve DPPH (8 mg) in ethanol (100 mL). A 50 L sample aliquot was mixed with a 100 L DPPH reagent. The mixture will then be incubated for 30 minutes at room temperature and in the dark. The absorbance will be measured at 517 nm using a microplate reader. All measurements were taken two times. A serial dilution at 1000, 500, 250, 125, 62.5, and 31.25 ppm was used in this assay. The IC50 calculation was achieved from the equation 1.1 where *AB* is absorption of control, *AS* is absorption of sample and *I*% is percentage of inhibition.

$$(I\%) = (AB - AS/AB) \times 100 \quad (1.1)$$

3.6. Total phenolic content

The total phenolic content of *K. galanga* extracts was determined using the Folin-Ciocalteu reagent, as described [13] with minor modifications. The Folin-Ciocalteu reagent (FCR) was diluted in distilled water (1:10) and thoroughly mixed. An aliquot (25 L) of each extract received 125 L of 10% Folin-Ciocalteu reagent. After swirling the mixtures for 5 minutes, they were set aside. The reaction mixtures were then treated with 125 L of 7.5 per cent sodium carbonate solution. The mixtures are incubated for 30 minutes at room temperature before their absorbances at 760 nm are measured against the reagent blank. As a reference standard, various concentrations of gallic acid (25 mg/L) were used. Every experiment was

conducted in duplicate. Total phenolic contents of the samples were determined from the calibration curve equation, y = = 0.0019x + 0.1066 with R2 = 0.9254 and were expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

4. Results and discussion

4.1. Extraction of K. galanga essential oil

The percentage yield for leaves and rhizomes oil of *K. galanga* with the pretreatment of UAE showed a higher percentage compared to the oil yield calculated from essential oil extracted without pretreatment (Table 1). Ultrasonic pretreatment affects the extraction yield at room temperature. Experimental results revealed that the temperature of 40 °C, solvent to sample ratio 5:1 and time (30 minutes) yielded 10.81 % essential oil. Higher essential oil extraction may be possible with ultrasonic pretreatment due to increased mass transfer by rupturing the cell wall, which increases product yield.

Table 1. Percentage yield for each extraction

Sample	Weight of sample (kg)	Weight of essential oil (g)	Percentage yield (%)
KGEO Rhizome with UAE	0.14	1.56	10.81
KGEO Rhizome	0.14	1.32	9.20
KGEP Leaves with UAE	0.10	0.81	9.69
KGEO Leaves	0.10	0.77	7.42

*KGEO: *Kaempferia galanga* essential oil

4.2. Identification of chemical constituent in K. galanga essential oil

GC and GC-MS analyzed the chemical constituent from leaves and rhizomes essential oil of *K. galanga*. Based on the analysis, ethyl p-methoxycinnamate (61.33%) and ethyl – cinnamate (15.54%) were analyzed as the major compound identified in the essential oil of rhizomes from *K. galanga* without pretreatment of UAE while ethyl p-methoxycinnamate (62.57%), and pentadecane (15.56%) were identified as major compound in essential of *K. galanga* with pretreatment of UAE. The composition of chemical constituent of rhizome and leaves of *K. galanga* is shown in Table 4.1 and Table 4.2. On the other hand, the major compound identified in the leaves part *of K. galanga* essential oil were ethyl p-methoxycinnamate (23.7%) and borneol (2.15%). Meanwhile, the major compound found in the essential oil of leaves in the *K. galanga* with pretreatment of UAE were identified as ethyl p-methoxycinnamate (24.52%), ethyl cinnamate (5.10%) and borneol (8.45%).The results showed that the oils contain a complex combination of numerous organic compounds, many of which were present in trace amounts. Because of that, it is worth concluding that the chemical composition of oils extracted from leaves and rhizomes varies greatly.

4.3. Determination of total phenolic content

Gallic acid has been used as a calibration reference. The TPC test is a calorimetric method that relies on electron transfer reactions between the Folin-Ciocalteu reagent and the phenolic component of the extract. The absorbance was measured using a microplate reader. When phenolic compounds react in the presence of Folin-Ciocalteu reagents, they produced more complex compounds. This is because the Folin Ciocalteu reagent contains sodium tungstate and sodium molybdate, thus complex compounds can be produced [14]. The reactions of these compounds produced a blue colour, indicating the presence of phenolic compounds in the sample. The maximum phenolic concentration was found in the essential oil of rhizomes in *K. galanga* (10.589 GAE/g). Figure 1 shows the calibration graph for standard solution.

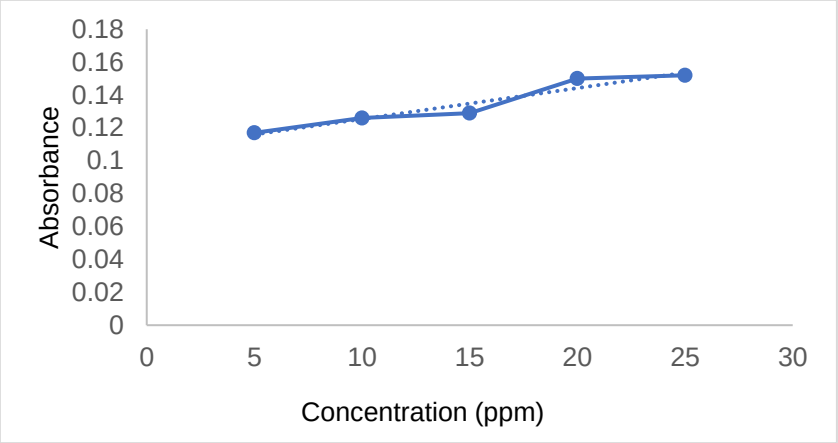

Figure 1. Calibration graph for standard solution

4.4. DPPH Assay

K. galanga rhizome with UAE pretreatment exhibits the antioxidant with an IC50 value of 128.91 mg/ml compared to other extracts that have a more significant value of IC50 (Table 2). Compared with gallic acid, it has IC50 of 47.58 mg/ml as DPPH positive control. *K. galanga* rhizomes have a greater value than gallic acid as the standard because it exhibits fewer phenolic compound in gcms analysis and it is confirmed to exhibit a weak antioxidative properties From the data above, a chemical composition such as benzene, eucalyptol, borneol, pentadecane, and ethyl p- methoxycinnamate are oxidative compounds shown to exist in the *K. galanga* rhizome Ultrasonic extraction pretreatment methods can also increase the antioxidant activity of *K. galanga* extract. This is due to the fact that ultrasonic waves was formed, which will be extracted so that the cell wall breaks in the material. It causes compounds to be released into the extract [14].The more components of the content that are extracted, the more antioxidant activity of *K. galanga* extract is increased.

Table 2. IC_{50} value for each extraction

Sample extract	IC_{50} value (µg/mL)
KGEO Rhizome with UAE pretreatment	128.91
KGEO Rhizome	175.64
KGEO Leaves with UAE pretreatment	209.12
KGEO Leaves	239.41
Gallic acid	47.58

Conclusion

Essential oil extraction of *K. galanga* rhizomes and leaves was successfully done using hydrodistillation and the UAE technique. The essential oil extracted later was used for the identification of the chemical composition of *K. galanga* essential oil. The major compound found in the essential oil of *K. galanga* rhizome is confirmed to be ethyl p- methoxycinnamate, followed by 2-propenoic acid and pentadecane. Meanwhile, major compounds identified in *K. galanga* leaves are ethyl p- methoxycinnamate, camphene and borneol. For total phenolic content, the determination of phenolic compound was conducted by using the Folin-Ciocalteu reagent. According to the data obtained, essential oil of *K. galanga* rhizome with UAE pretreatment exhibits the greatest TPC value at 10.589 mg GAE/g. For the antioxidant analysis of *K. galanga* leaves and rhizomes extract, DPPH radical scavenging was conducted. For the DPPH assay, it is expected that rhizomes of *K. galanga* have the lowest IC_{50} value, which is 128.91µg/mL followed by the IC_{50} value of 175.64 µg/mL, 209.12 µg/mL, and 239.41 µg/mL respectively. The lower the IC_{50} value indicates that the fraction has better antioxidant properties. Essential oil extract of *K. galanga* rhizomes has higher antioxidant activity compared to leaves essential oil extract. Based on the TPC test of *K. galanga*, it is found that phenolic compounds are present in both rhizomes and leaves essential oil extract.

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