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RESEARCH ARTICLE

Physicochemical and Biological Activities of *Alpinia galanga* (L.) Willd. Essential Oil Extracted by Pulsed Electric Field and Ultrasonication Pretreatments of Hydrodistillation

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Abstract

The *Alpinia* genus has long been used in many countries as a traditional medicine and is known for its antioxidant and antimicrobial properties. Galangal essential oils (GEO) increase the therapeutic potential of various *Alpinia* species. However, the extraction of high-quality GEO is challenging because of its low yield and inconsistent quality. This study investigated alternative pre-treatment methods before hydrodistillation of *Alpinia galanga* (L.): Pulsed electric field (PEF) with parameters 3000 V, 5000 Hz for 30 min and ultrasonication with parameters 50 W/m, A = 40%, for 30 min with the addition of distilled water 1:1 (v/m) Willd. PEF pre-treatment resulted in the highest GEO yield (0.88% ± 0.11%) and a greater proportion of 1,8-cineole (25.01%) compared to the control and ultrasonication pre-treatment. Furthermore, PEF significantly enhanced antioxidant activity, as shown by DPPH (41.88 ± 0.07 µg/mL) and ABTS (46.43 ± 0.02 µg/mL) assays. Both PEF and ultrasonication improved antimicrobial activity against *Escherichia coli* than *Staphylococcus aureus*, with PEF demonstrated the highest inhibitory activity against *Aspergillus niger* compared to *Saccharomyces cerevisiae*. These results suggest that PEF is an effective green pre-treatment method fpr enhancing both yield and bioactivity of GEO form *A. galanga* (L.) Willd., highlighting its potential for application in the food and pharmaceutical industries.

Keywords: Galangal essential oil, Pulsed electric field, Ultrasonication, Hydrodistillation, Antimicrobial, Antioxidant

Introduction

Essential oils have a wide variety of biological activities [1], including antioxidant [2], and antimicrobial properties [3]. The demand for essential oils and their derivatives has recently increased [4,5], driven by a growing preference for natural compounds and the desire to minimize the side effects associated with synthetic chemicals [6]. GEO is an essential oil that contain bioactive compounds such as 1,8-cineole [7-10] that exhibits significant anti-inflammatory, antioxidant, antimicrobial, and antifungal activities [5,11-17]. Therefore, the food and pharmaceutical industries have integrated GEOs into their products [3,18-21] as natural antioxidants and antibacterial agents, especially in the active packaging of fruits [22,23]. Galangal is one of the most widely produced rhizome biopharmaceutical plants in Indonesia [24], after ginger and turmeric, this abundance indicates a significant potential for commercial use.

However, owing to low yields and quality variations during extraction [25-27], researchers have explored alternative pre-treatment methods, including PEF and ultrasonication, to obtain high-quality GEO. These non-thermal methods are safe and effective for increasing the yield and quality of essential oils without

damaging cellular components and, offer cost-effective and sustainable advantages [28-30]. Previous studies have shown that supercritical fluid CO_2 extraction pre-treatment can increase the yield of *A. officinarum* essential oil compared to extractions without pre-treatment [19]. Similarly, PEF pre-treatment has been studied to increase the yield of *A. purpurata* K. Scumm. essential oil by 0.3% - 0.52% [31], and other PEF studies have investigated PEF pre-treatment for extracting oils from sunflower seed [32], rapeseed [33], and various plants before hydrodistillation [27,34], including coriander seeds [35], as well as for the extraction of phytoconstituents [36].

Ultrasonication pre-treatment has also been shown to improve extraction by combining the benefits of ultrasonication with hydrodistillation [37-39]. Ma *et al.* [40] were used as references for the ultrasonication conditions in this study, and previous studies on ultrasound-assisted extraction of bioactive compounds from commercial crops [26], tarragon essential oil [38], isoflavone extraction from kudzu (*Pueraria lobata* Ohwi) root waste [41], and rice bran oil extraction [42].

Among *Alpinia* species, *A. galanga* is widely used in cuisine, particularly in Southeast Asia, where its aromatic properties contribute to food flavor. Traditionally, *A. galanga* has been used to treat colds, stomach pain and discomfort, edema, bronchitis, and chest pain. However, providing GEO for therapeutic applications remains a challenge because of low extraction yields. Hydrodistillation is the most commonly used extraction method for GEO. Pre-treatment to disrupt plant cells before extraction may improve GEO extractability. In particular, the application of novel technologies, such as PEF and ultrasonication as pre-treatments for GEO extraction from *A. galanga* (L.) Willd. has not been reported.

This study aimed to evaluate the oil yield, composition, and biological activities of GEO obtained from *A. galanga* (L.) Willd. using PEF and ultrasonication pre-treatments before hydrodistillation. This research will contribute to the scientific knowledge of the green extraction of natural products and assess the potential of *A. galanga* in food, beverage, and pharmaceutical applications. The novelty of this study is the investigation of the effects of pre-treatment methods before hydrodistillation on the quantity and quality of essential oils from *A. galanga* (L.) Willd.

Materials and methods

Materials

Galangal rhizomes, *A. galanga* (L.) Willd. were obtained from Sumenep, Indonesia. The following analytical-grade chemicals were used: *n*-hexane (>99% purity; Merck), double-distilled water (ddH_2O), ethyl alcohol (Sigma-Aldrich), potassium hydroxide (KOH), hydrochloric acid (HCl), sodium hydroxide (NaOH), *phenolphthalein* indicator, sulfuric acid (H_2SO_4), and toluene. The equipment used included: Knives, glassware, analytical balance (Denver Instrument), Soxhlet apparatus (Schott Duran), a water bath, and distillation equipment. Gas chromatography-mass spectrometry (GC-MS, Shimadzu GC-2030), UV-Vis spectrophotometry, microplate reader (ELx800), PEF chamber and ultrasonic processor (Hielscher UP200St).

Sample preparations

Fresh galangal rhizomes, *A. galanga* (L.) Willd. were obtained from local farmers in Sumenep, Indonesia. The Rhizomes, harvested at 12 months old, 5 - 10 cm in length and 3 - 5 cm in diameter, with a moisture content of 80% ± 5%. The Rhizomes were cleaned by washing thoroughly with water to remove dirt and soil, followed by draining. The cleaned rhizomes were then sliced thinly (3 - 5 mm thickness) and dried at 40 °C for 5 h using a food dehydrator. The dried slices were ground into a fine powder.

PEF and ultrasonication pretreatment

PEF pre-treatment was conducted at 3000 V and 5000 Hz for 30 min [31]. The cathode-anode distance can be adjusted from 5 to 40 cm, and the chamber is made of acrylic with a diameter of 12 cm and a thickness of 0.2 mm. Ultrasonication pre-treatment with modification [40] was conducted with parameters 50 W/m, A = 40%, for 30 min with the addition of distilled water 1:1 (v/m).

Extraction by hydrodistillation

Following pre-treatment, the fine powder of galangal rhizome (2000 - 4000 g) was distilled by hydrodistillation using a Clevenger-type apparatus. Distillation was carried out for 4 h at 100 - 110 °C, following a modified procedure [31]. This process facilitated the release of essential oils from galangal rhizomes. During the distillation process, the volatile oil

components were carried by steam to a condenser. Condensation of the steam and oil vapor mixture occurs in the condenser, resulting in a collected liquid mixture. The condensed mixture was then separated, and the oil phase was dehydrated using anhydrous sodium sulfate (Na_2SO_4). The aqueous phase (distilled water) was then removed and collected separately.

Essential oil yield

The yield of GEO yield was determined gravimetrically. The oil collected from each extraction (hydrodistillation only, PEF pre-treatment followed by hydrodistillation, and ultrasonication pre-treatment followed by hydrodistillation) was measured, and the yield was calculated as a weight percentage (w/w) based on 100 g of dry galangal powder. The results are reported as %(w/w) [14].

$$\%\, Yield = \frac{the\ weight\ of\ the\ essential\ oil\ (g)}{the\ weight\ of\ raw\ materail\ (g)} \times 100 \quad (1)$$

Gas Chromatography-Mass Spectrometry (GC-MS)

The volatile components of the GEO were identified using gas chromatography-mass spectrometry (GC-MS) following a modified method [43]. Zero point eight µL sample of the essential oil, diluted in ethanol, was injected in split mode (10:1) into the GC-MS system (Shimadzu GC-2030). Helium was used as the carrier gas at an inlet pressure of 53.5 kPa. The carrier gas flow rate were 1.00 mL/min through the primary column and 2.00 mL/min through the secondary column. Separation of the volatile compounds was achieved using an SH-5MS capillary column (30 m×0.25 mm×0.25 µm film thickness, Shimadzu). The oven temperature program was as follows: Initial temperature of 50 °C (held for 2 min), ramped at 5 °C/min to 250 °C, and held at 250 °C for 18 min. The injection temperature was 250 °C. Identification of the volatile components was performed by comparing their retention indices (relative to a homologous series of C_8-C_{20} alkanes on the SH-5MS column) with literature values and by matching their mass spectra against the NIST 20 MS and Wiley libraries. The percentage composition of the essential oil was determined by normalizing the peak areas of the total ion chromatogram.

Physicochemical characterization

Specific gravity

The specific gravity of each GEO sample was determined using a pycnometer following the method described for liquid fats and oils [44]. A calibrated pycnometer was filled with GEO at 25 °C, and the weight of the GEO was measured. Specific gravity was calculated using an appropriate formula. All assays were performed in triplicates.

Viscosity

The viscosity of each GEO sample was measured in triplicate using a Brookfield LVT rotational viscometer equipped with a thermostatic bath for temperature control [45]. An LV-4 spindle (no. 64) was used. For each measurement, 10 mL of GEO was placed in the viscometer sample chamber and the viscosity was measured at 25 °C.

Moisture

The moisture content of each GEO sample was determined using the Karl Fischer titration method [44]. GEO (5-25 g) was weighed and placed in a titration vessel containing ≤100 mg of water in anhydrous chloroform ($CHCl_3$) and methanol. The mixture was then titrated with Karl Fischer reagent (either undiluted or diluted as needed) to an electrometric endpoint. Blank titration was performed using the same amounts of reagents, diluents, and solvents but without the GEO sample.

Ester number

GEO (2 g) was accurately weighed and placed in a 125 mL separatory funnel. Ethanol (8 mL) was added, followed by a few drops of the phenolphthalein indicator. The mixture was neutralized by the addition of NaOH solution until a pink color appeared. Zero point five N KOH in ethanol (25 mL) was added and the mixture was shaken until the pink color disappeared. The solution was refluxed for 1 h. After cooling, the excess base was titrated with 0.25 N sulfuric acid (H_2SO_4) until the pink color reappeared. Blank titration was performed using the same procedure but without the GEO sample. The ester number was calculated using the following formula [44]:

$$Ester\ number = \frac{(V_{blank} - V_{sample}) \times N_{H_2SO_4} \times 56.1}{m} \quad (2)$$

where:
V_{blank} = Volume of H_2SO_4 used in the blank titration (mL).
V_{sample} = Volume of H_2SO_4 used in the sample titration (mL).
$N_{H_2SO_4}$ = Normality of H_2SO_4.
56.1 = Molecular weight of KOH.
m = Mass of GEO sample (g).

Acid number

GEO (2 g) was accurately weighed and placed in a 250 mL conical flask. Freshly neutralized hot ethanol (8 mL) and phenolphthalein indicator solution (5 drops) were added. The mixture was heated to boiling for approximately 5 min and then titrated while still hot with 0.2 N KOH in ethanol. The solution was vigorously shaken during titration. The acid number was calculated using the following formula [44]:

$$Acid\ number = \frac{56.1 \times V_{KOH} \times N_{KOH}}{m} \quad (3)$$

where:
V_{KOH} = Volume of KOH used in the titration (mL).
N_{KOH} = Normality of KOH.
56.1 = Molecular weight of KOH.
m = Mass of GEO sample (g).

Antioxidant activity

The antioxidant activity of the GEO was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay [14] and the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay [46].

DPPH assay

Twenty µL of each GEO sample (at concentrations of 1, 5, 10, 20, 50, and 100 µg/mL in ethanol) or Trolox (used as a positive control) in ethanol was added separately to 130 µL of a 0.1 mM DPPH solution in ethanol in a 96-well microplate. After incubation in the dark for 30 min at 25 °C, the absorbance of each mixture and control (containing DPPH solution and ethanol only) was measured at 517 nm using ethanol as the blank. The IC_{50} value (the concentration required to scavenge 50% of DPPH radicals) was determined from a graph plotting the percentage inhibition against the GEO concentration. The percentage DPPH radical scavenging activity was calculated using the following formula:

$$\%\ \text{DPPH Scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

ABTS assay

The ABTS stock solution (7.0 mM) was mixed with an equal volume of potassium persulfate ($K_2S_2O_8$) solution (2.45 mM) and incubated in the dark at room temperature for 14 h. The resulting ABTS solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. In a 96-well microplate, 130 µL of diluted ABTS solution was added to 20 µL of each GEO sample (at concentrations of 1, 5, 10, 20, 50, and 100 µg/mL in ethanol) or Trolox (positive control) in ethanol (Duan *et al.*). After incubation in the dark for 6 min, absorbance was measured at 734 nm using ethanol as the blank. The IC_{50} value (the concentration required to scavenge 50% of ABTS radicals) was determined from a graph plotting the percentage inhibition against the GEO concentration. The ABTS radical scavenging activity was calculated using the following formula:

$$\%\ \text{ABTS Scavenging} = 1 - \frac{A_{sample}}{A_{control}} \times 100 \quad (5)$$

Antimicrobial activity

The antimicrobial activity of GEOs was evaluated against gram-positive bacteria (*S. aureus*), gram-negative bacteria (*E. coli*), yeast (*S. cerevisiae*), and mold (*A. niger*) using, and a modified agar well diffusion method [14]. Bacterial cultures: *S. aureus* and *E. coli* were streaked onto nutrient agar (NA) plates using a sterile inoculation loop and incubated at 37 °C for 24 h. Yeast and mold cultures: *S. cerevisiae* was streaked onto yeast extract agar (YEA) plates, and *A. niger* was streaked onto potato dextrose agar (PDA) plates and incubated at 27 °C for 72 h.

Five µL of GEO (25, 50, and 100 µg/mL) or gentamicin as a positive control for bacteria (10 µg/mL) or potassium sorbate (25, 50, and 100 µg/mL) as a positive control for *S. cereviceae* and *A. niger* (25, 50, and 100 µg/mL) were loaded into their respective labelled wells. The plates were incubated under the

same conditions described above. After further incubation, the plates were uncovered and the diameters of the zones of inhibition were measured in millimeters (mm). All analyses were performed in triplicates.

Statistical analysis

The experiments (yield analysis, antioxidant activity, and physicochemical characterization) were conducted in triplicate, and the results are expressed as the mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences between treatments at a significance level of $\alpha = 0.05$. Means were compared using the least significant difference (LSD) test with XLSTAT software (version 17.06). Differences were considered statistically significant at a p-value of <0.05.

Results and discussion

Essential oil yield

Volatile oil was extracted from dried *A. galanga* rhizomes using 2 distinct pre-treatment methods. Quadruplicates extractions yielded average oil contents of 0.52 %(w/w) for the non-pre-treated samples, 0.88 %(w/w) for samples subjected to PEF pre-treatment, and 0.65 %(w/w) for samples subjected to ultrasonication (**Table 1**). Statistical analysis revealed a significant effect of pre-treatment on oil yield ($p < 0.05$). Comparable studies from various regions report *A. galanga* rhizome oil yields ranging from 0.11 to 0.93 %(w/w) [10,14,47-51].

Table 1 Yield comparison of *A. galanga* rhizome essential oil with previous studies.

Pre-treatment	% yield		
	HD	**PEF-HD**	**Ultrasonication-HD**
This study	0.52 ± 0.03[a]	0.88 ± 0.11[b]	0.65 ± 0.23[c]
A. galanga (L.) Willd [10]	0.31		
A. galanga (L.) Willd [48]	0.11		
A. galanga (L.) Willd [49]	0.93		
A. galanga (L.) Willd [50]	0.27		
A. purpurata, K. Scumm [31]		0.34	
A. officinarum [51]			5.01

Data expressed as means ± SD, n = 4.
HD = Hydro-distillation; PEF-HD = Pulsed electric field - hydrodistillation.

The results indicate that PEF pre-treatment significantly enhances the extraction yield compared to traditional hydrodistillation and ultrasonication pre-treatment. PEF is a novel, nonthermal intensification method known to improve extraction efficiency, reduce extraction time, increase yield, and lower energy consumption [32,33]. Similarly, ultrasonication has been shown to increase the extraction efficiency of diverse phytochemicals, including essential oils [41,52]. Both PEF and ultrasonication are considered green extraction techniques, aligning with the growing emphasis on sustainable and environmentally friendly methods, which can potentially reduce energy costs by shortening the extraction times for equivalent essential oil yields [53]. Previous studies indicate that applying PEF at higher voltage (e.g. 2 - 20 kV/cm) and optimized pulse frequency enhances membrane permeabilization and improves mass transfer in extraction processing [54]. Similarly, precise control over ultrasonication duration and amplitude yields significant improvements in extraction efficiency via cavitational disruption, though excessive time may degrade heat-sensitive components [55,56].

While traditional hydrodistillation remains a common method for essential oil extraction, alternative techniques such as supercritical CO_2 and solvent extraction offer distinct advantages and disadvantages. Supercritical CO_2 extraction, for instance, yields high-purity oils but is associated with significant capital and operating costs [57]. Solvent extraction, which is

capable of high yields, raises concerns about potential solvent residues in the final product. In contrast, PEF and ultrasonication are greener alternatives because of reduced solvent use and lower energy consumption. By utilizing PEF, PEF selectively disrupts cell membranes, resulting in enhanced extraction yields and reduced energy consumption [58]. Ultrasonication, through the application of high-frequency sound waves, promotes cell wall disruption and mass transfer and contribute to improved extraction efficiency [55].

Furthermore, supercritical CO_2 extraction, although effective, has limitations related to solvent recovery and the cost of supercritical fluid generation. By contrast, PEF and ultrasonication rely minimally on solvents, thereby offering a greener alternative that aligns with contemporary trends towards sustainable practices in the food and pharmaceutical industries [59]. The adoption of these methods can provide economic benefits by reducing operating costs, while enhancing the appeal of products through the production of higher-quality extracts, which include fewer unwanted compounds and better preservation of volatile components.

Chemical composition of the GEO

This study demonstrated that different pre-treatment methods resulted in variations in the chemical composition of the essential oil (**Table 2**). Thirty-eight identified compounds were characterized by hydrodistillation without pre-treatment, 35 by PEF, and 21 compounds were characterized by ultrasonication pre-treatment. The highest relative constituent percentage was 25.01% for 1,8-cineole in PEF pre-treatment, followed by 13.61% in ultrasonication pre-treatment, and 8.83% in control. The identified constituents were β-pinene, fenchyl acetate, α-pinene, camphene, α-terpineol, linalool, terpinene-4-ol, camphor, methyl cinnamate, α-myrcene, fenchone, and fenchol (**Table 3**). Consistent with previous literature, 1,8-cineole was found to be the major constituent of GEO, followed by β-pinene, camphene, α-pinene, α-terpineol, and camphor [14,51,60].

Variations in galangal species, sources, and origins influence the chemical composition of the extracted essential oil. **Table 1** compared the percentage yields of GEO from various *Alpinia* species with different origins and sources, meanwhile **Table 3** compared the chemical composition of this study with the previous study [14], who used dried *A. galanga* rhizomes from the Khurda district of Odisha, India.

Table 2 Chemical composition of essential oil of *A. galanga* (L.) Willd.

RT[a] (minute)	Compound	Relative (%)[b]		
		HD	PEF-HD	Ultrasonication-HD
4.935	Furfural	-	-	1.90 ± 0.10
6.298	Styrene	-	1.50 ± 0.28	-
6.306	Styrene	-	-	0.48 ± 1.45
7.240	α-Thujene	0.33 ± 0.71	-	-
7.388	α-Pinene	4.61 ± 0.57	-	14.60 ± 0.86
7.578	trans-β-Ocimene	-	10.88 ± 0.56	-
7.882	Camphene	0.53 ± 1.54	7.05 ± 0.28	7.30 ± 1.56
8.218	Benzaldehyde	-	0.77 ± 0.42	-
8.256	Benzaldehyde	-	-	-
8.607	β-Pinene	3.21 ± 0.42	11.59 ± 2.12	16.22 ± 2.65
9.091	β-Myrcene	1.15 ± 1.37	2.59 ± 4.32	-
9.393	α-Phellandrene	-	0.31 ± 0.28	-
9.687	α-Terpinene	-	0.33 ± 0.56	-
10.775	β-Ocimene	-	1.54 ± 0.26	-

RT[a] (minute)	Compound	Relative (%)[b]		
		HD	PEF-HD	Ultrasonication-HD
10.949	1,8-Cineole	8.83 ± 0.57	25.01 ± 1.94	13.61 ± 1.45
11.095	γ-Terpinene	-	1.13 ± 0.68	-
11.335	Acetophenone	-	0.37 ± 0.28	-
12.086	α-Para-dimethylstyrene	1.64 ± 2.06	-	-
12.251	L-Fenchone	-	6.03 ± 0.42	8.47 ± 0.65
12.750	Fenchol	-	1.99 ± 0.28	2.61 ± 0.32
12.291	Linalool	0.34 ± 0.42	2.68 ± 0,22	1.24 ± 0.68
12.983	2-Cyclohexen-1-ol. 1-methyl-4-(1-methylethen	0.40 ± 0.71	-	-
13.129	Fenchol	-	-	2.61 ± 0.42
13.705	(+)-2-Bornanone	0.22 ± 1.42	-	-
13.720	d-Camphor	-	5.81 ± 0.28	-
13.941	(+)-2-Bornanone	-	-	3.17 ± 0.64
14.038	Isoborneol	-	0.86 ± 0.22	-
14.394	δ Terpineol	0.53 ± 0.12	-	-
14.751	Terpinen-4-ol	3.46 ± 0.42	1.07 ± 0.26	-
15.104	α-Terpineol	1.82 ± 0.52	1.83 ± 0.28	3.33 ± 0.28
15.249	Estragole	1.01 ± 1.22	-	-
15.535	n-Octanyl acetate	0.25 ± 1.64	15.54 ± 0.86	-
15.838	(-)-trans-Isopiperitenol	0.23 ± 0.57	15.84 ± 1.12	-
15.804	α-Fenchyl acetate	-	0.91 ± 0.22	1.75 ± 0.86
17.916	Tridecane	1.03 ± 0.71	-	-
19.770	Carveol acetate	1.62 ± 0.12	-	-
20.378	Geranyl acetate	4.14 ± 0.57	-	-
21.160	Methyl cinnamate	-	0.63 ± 0.28	-
21.425	Caryophyllene	4.22 ± 0.42	-	-
21.616	cis-α-Bergamotene	1.06 ± 0.26	-	-
22.675	Humulene	-	2.03 ± 4.42	-
22.788	β.-Farnesene	7.86 ± 3.42	-	-
23.181	β-Selinene	-	-	3.72 ± 2.66
23.238	β-Selinene	-	1.55 ± 4.64	-
23.742	γ-Muurolene	-	3.63 ± 5.86	-
23.469	Hexadecane	6.42 ± 0.57	-	-
23.676	γ-Cadinene	-	-	2.71 ± 1.45
23.711	β-Bisabolene	3.91 ± 4.12	-	-
23.888	δ-Cadinene	-	2.67 ± 6.16	-
24.810	Nerolidyl acetate	2.46 ± 0.71	-	-
25.480	Caryophyllene oxide	3.61 ± 2.42	0.83 ± 0.28	1.65 ± 0.44

RT[a] (minute)	Compound	Relative (%)[b]		
		HD	PEF-HD	Ultrasonication-HD
26.037	Tetramethyl-12-oxabi	2.41± 4.14	0.47 ± 0.12	0.46 ± 2.65
26.515	Cadinol	-	0.62 ± 4.62	-
26.841	α-Cadinol	-	1.20 ± 0.28	-
26.980	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethylde	-	0.69 ± 1.82	-
27.432	(1-Acetoxyallyl) phenyl acetate	6.56 ± 4.18	-	-
27.889	3-Heptadecene	7.49 ± 0.89	-	-
28.219	Pentadecanal	1.37 ± 0.57	-	-
28.949	2,6,10-Cycloundecatrien-1-one, 2,6,9,9-tetram	1.23 ± 5.42	-	-
30.979	2,6,10-Dodecatrien-1-ol	5.58 ± 0.71	-	-
31.657	Nonadecanol-1	2.96 ± 4.35	-	-
32.942	Eicosyl acetate	0.92 ± 0.86	-	-
33.271	n-Hexadecanoic acid	1.62 ± 4.40	0.39 ± 0.38	-
34.338	1,6,10,14,18,22-Tetracosahexaen-3-ol	0.56 ± 0.10	-	-
33.383	Linoleyl acetate	0.51 ± 0.30	-	-
36.023	n-Hexadecanoic acid	-	-	0.80 ± 0.68
36.259	9,12-Octadecadienoic acid (Z.Z)-	-	0.59 ± 1.42	0.83 ± 1.45
37.376	Z-13-Octadecen-1-yl acetate	0.48 ± 1.71	-	-
43.165	Bis(2-ethylhexyl) phthalate	-	2.04 ± 3.28	-
Total Identified		**95.70 ± 2.56**	**98.22 ± 0.82**	**97.81 ± 0.95**

[a]Retention time; [b]Relative area percentage of the peak; data expressed as mean ± SD, n = 3.
HD = Hydro-distillation; PEF-HD = Pulsed electric field - hydrodistillation.

This study revealed that PEF and ultrasonication pre-treatment of galangal rhizomes resulted in distinct volatile compound and phytochemical profiles compared with hydrodistillation alone. Although some components were reduced or absent due to pre-treatments, this did not negatively impact the overall essential oil composition [34,35,53]. Furthermore, the abundance, diversity, and potential synergistic effects of bioactive compounds in GEO suggest enhanced antimicrobial and antioxidant properties. However, 1,8-cineole remained one of the dominant constituents in all treatment groups. These variations may be attributed to differences in cell disruption mechanisms between PEF and Ultrasonication, which influence the release and preservation of volatile compounds. These variations may be attributed to differences in cell disruption mechanisms between PEF and Ultrasonication, which influence the release and preservation of volatile compounds.

PEF and ultrasonication differ in their operational mechanisms and effects on essential oil extraction. PEF involves the application of short, high-voltage electric pulses to plant material, leading to the electroporation of cell membranes and the release of intracellular

compounds, including essential oils [36]. Ultrasonication uses high-frequency sound waves to disrupt cell walls and enhance mass transfer, thereby promoting essential oil extraction [42].

Table 3 Comparison of *A. galanga* (L.) Willd. essential oil chemical composition with previous studies.

Component	Relative %			Singh *et al.* [10] (g/100 g)
	HD	PEF-HD	US-HD	
1,8 Cineole	8.83	25.01	13.61	34.31
β-Pinene	3.21	11.59	16.22	9.69
Fenchyl acetate		0.91	1.75	8.71
α-Pinene	4.61	-	14.6	5.69
Camphene	0.53	7.05	7.3	5.89
α-Terpineol	1.82	1.83	3.33	1.05
Linalool	0.34	2.68	1.24	-
Terpinen-4-ol	3.46	1.07	-	0.11
Camphor	-	5.81	-	4.51
Methyl cinnamate	0.63	-	-	2.35
α-Myrcene	1.15	2.59	-	0.81
Fenchone	-	6.03	8.47	0.51
Fenchol	-	1.99	2.61	0.39

HD = Hydro-distillation; PEF-HD = Pulsed electric field - hydrodistillation.

Physicochemical characteristics

Table 4 presents the physicochemical characteristics of GEO in this study. The physical and chemical properties of GEO obtained using different pre-treatment methods showed no significant differences ($p < 0.05$). These characteristics can be used to assess GEO quality in comparison with the relevant essential oil standards and customer requirements.

Table 4 Physical and chemical characterisation of *A. galanga* essential oil.

No	Parameter	Value*		
		HD	PEF-HD	US-HD
	Physical			
1	Specific gravity (g/mL)	0.92 ± 0.02[a]	0.93 ± 0.002[a]	0.92 ± 0.002[a]
2	Viscosity (cPa)	9.37 ± 0.03[a]	9.69 ± 0.03[a]	9.91 ± 0.01[a]
	Chemical			
1	Moisture (%)	0.20 ± 0.13[a]	0.19 ± 0.09[a]	0.20 ± 0.12[a]
2	Ester number (mg KOH/g)	10.45 ± 0.41[a]	11.45 ± 0.24[b]	11.34 ± 0.32[b]
3	Acid number (mg KOH/g)	1.38 ± 0.06[a]	1.02 ± 0.07[a]	1.01 ± 0.05[a]

*Data are expressed as the means ± SD, n = 3.

HD = Hydro-distillation; PEF-HD = Pulsed electric field - hydrodistillation.

Specific gravity measurements revealed that the apparent viscosity of GEO does not directly correlate with its density or specific gravity. GEO from PEF pre-treatment exhibited the highest specific gravity (0.93 g/mL), followed by GEO from hydrodistillation (0.92 g/mL) and ultrasonication pre-treatment (0.92 g/mL).

Conversely, GEO from PEF pre-treatment, despite having the highest specific gravity, displayed the lowest viscosity (9.69 cPa), while GEO from ultrasonication pre-treatment showed a viscosity of 9.91 cPa. GEO from hydrodistillation without pre-treatment had a viscosity of 9.37 cPa. Overall, the specific gravity and viscosity values of GEO are consistent with the characteristics of natural essential oils [61].

The moisture content was analyzed to determine the amount of water present during distillation. GEO obtained from hydrodistillation and ultrasonication pre-treatment showed similar moisture content (0.2%), while GEO from PEF pre-treatment had a moisture content of 0.19%. These variations in moisture content can be attributed to hydrolysis and variations in the amount of water present in the distillation apparatus during different pre-treatment methods [62].

mg.KOH/g followed by GEO from PEF pre-treatment (11.45 mg KOH/g), GEO from ultrasonication pre-treatment (11.34 mg KOH/g) and hydrodistillation alone (10.45 mg KOH/g). The ester number of PEF was significantly higher than that of hydrodistillation ($p < 0.05$), but not significantly higher than that of ultrasonication by one-way ANOVA. Lower water contact during pre-treatment resulted in GEO with a higher ester content. All GEO samples exhibited low acid values, with the lowest acid value observed in GEO from ultrasonication pre-treatment (1.01 mg KOH/g), followed by GEO from PEF pre-treatment (1.02 mg KOH/g), and the highest acid value in GEO control (1.38 mg KOH/g). The increase in acid number can be attributed to aldehyde oxidation, ester hydrolysis, and oil storage conditions. In this study, the samples were immediately dried with anhydrous sodium sulfate, stored in dark bottles in a refrigerator, and analyzed promptly to minimize oxidation and hydrolysis. Therefore, variations in acid and ester values may be influenced by distillation conditions, such as the longer contact time of GEO with water during ultrasonication pre-treatment compared to PEF pre-treatment and hydrodistillation.

Antioxidant activity

This study evaluated the antioxidant activity of GEO using DPPH and ABTS free radical scavenging assays (**Table 5**). GEO obtained from PEF pre-treatment exhibited significantly higher scavenging activity than the Trolox standard in both assays. Specifically, GEO from PEF pre-treatment demonstrated higher scavenging activity in the DPPH assay (41.88 ± 0.07) and the ABTS assay (46.43 ± 0.02) compared to the Trolox standard (16.82 µg/mL). Although numerically lower, neither ultrasonication pre-treatment nor hydrodistillation alone showed a statistically significant difference in scavenging activity compared to PEF ($p > 0.05$). The presence of phenolic compounds in GEO contributes to its antioxidant activity and enhances the free-radical scavenging ability of volatile oils [63]. The antioxidant activity of GEO can be increased by increasing the number of pulses in PEF pre-treatment, which specifically minimizes the loss of phenolic compounds from plant matrices during distillation [34,35].

Table 5 Antioxidant activity of *A. galanga* essential oil.

Treatment	IC_{50} (µg/mL)	
	DPPH	ABTS
Hydro = Distillation	55.49 ± 0.05[a]	58.67 ± 0.32[a]
PEF-HD	41.88 ± 0.07[b]	46.43 ± 0.02[b]
Ultrasonication-HD	49.45 ± 0.03[c]	52.36 ± 0.06[c]
Trolox	16.82 ± 0.06[d]	17.64 ± 0.14[d]

Data expressed as means ± SD, n = 3.

HD = Hydro-distillation; PEF-HD = Pulsed electric field - hydrodistillation.

These results suggest that 1,8-cineole, a major component of *A. galanga*, plays a significant role in antioxidant activity [64,65], antimicrobial properties, and has been recognized as a permeation enhancer [23]. Based on the findings of this study, it is evident that the secondary metabolites of galangal possess antimicrobial and antioxidant potential for application in food and medicinal products, such as GEO-based nanoemulsions [14,66], active food packaging [67], clinical anesthetics, and slimming aromatherapy [68].

Antimicrobial activity

Two bacterial species were selected to determine the antimicrobial activity of GEO by measuring the inhibition zone diameter (IZD) at specific concentrations (**Table 6**). The bacterial strains tested were gram-positive *S. aureus* and gram-negative *E. coli*, along with the fungal strains *A. niger* and *S. cerevisiae*. For bacterial strains, GEO from hydrodistillation exhibited the highest inhibitory activity against *S. aureus* (20.33 ± 0.58 mm), while GEO from PEF and ultrasonication pre-treatments showed the highest inhibitory activity against *E. coli*.

All antimicrobial tests on the bacterial strains were compared using gentamicin as a positive control. The variations in activity among GEO samples may be attributed to differences in the chemical composition of the essential oils resulting from various extraction pre-treatments. The chemical composition of essential oils influences the permeability of bacterial cell membranes [69,70]. The primary difference between *S. aureus* and *E. coli* is their cell wall composition. *S. aureus* possesses a thicker peptidoglycan layer, whereas *E. coli* possesses a thinner peptidoglycan layer and a complex lipopolysaccharide layer. This complexity hinders the penetration of bioactive compounds into *E. coli* cell walls [14]. Variations in the composition of the bioactive compounds also affect the level of bacterial inhibition. GEO produced from PEF and ultrasonication pre-treatments, which contain higher concentrations of 1,8-cineole than hydrodistillation alone, demonstrated more effective inhibition of *E. coli* than *S. aureus*. This suggests that 1,8-cineole is more effective in inhibiting *E. coli*.

Table 6 Antimicrobial activity *A. galanga* essential oil.

Treatment	Inhibition zone diameter (mm)			
	S. aureus	***E. coli***	***S. cerevisiae***	***A. niger***
Hydro-distillation				
25 ug/mL	12.33 ± 1.53	10.33 ± 0.58	11.33 ± 0.58	13.00 ± 0.00
50 ug/mL	17.67 ± 0.58	14.33 ± 0.58	15.33 ± 0.58	14.33 ± 0.58
100 ug/mL	20.33 ± 0.58	18.00 ± 0.00	19.00 ± 0.00	18.00 ± 0.00
PEF-HD				
25 ug/mL	8 ± 1	17.67 ± 0.58	11.67 ± 0.58	14.33 ± 0.58
50 ug/mL	15.33 ± 0.58	22.00 ± 0.00	15.67 ± 1.15	20.67 ± 0.58
100 ug/mL	17.67 ± 0.58	23.67 ± 0.58	17.67 ± 0.58	22.33 ± 0.58
Ultrasonication-HD				
25 ug/mL	8.67 ± 1.53	19.67 ± 0.58	10.33 ± 0.58	14.33 ± 0.58
50 ug/mL	18.67 ± 0.58	21.67 ± 1.53	13.33 ± 0.58	16.67 ± 0.58
100 ug/mL	19.67 ± 0.58	23.00 ± 1.00	15.00 ± 0.00	17.33 ± 0.58
Gentamicin				
10 ug/mL	22 ± 0	22.00 ± 0.00	-	-

Treatment	Inhibition zone diameter (mm)			
	S. aureus	***E. coli***	***S. cerevisiae***	***A. niger***
Pottasium sorbate				
25 ug/mL	-	-	10.33 ± 0.00	12.67 ± 0.58
50 ug/mL	-	-	14.67 ± 0.58	16.33 ± 0.00
100 ug/mL	-	-	18.33 ± 0.58	20.33 ± 0.58

Data expressed as means ± SD, n = 4.

For fungal strains, GEO from hydrodistillation showed the highest inhibitory activity against *S. cerevisiae*, where GEO from PEF pre-treatment exhibited the highest inhibitory activity against *A. niger*. All antimicrobial tests on fungal strains were compared with potassium sorbate ($C_6H_7KO_2$) as a positive control, a preservative with antibacterial, anti-yeast, and antifungal properties.

The antimicrobial activity of GEO may be attributed to a combination of bioactive compounds, with 1,8-cineole as the predominant constituent. GEO from PEF pre-treatment, containing 25.01% 1,8-cineole, demonstrated high inhibitory activity against *E. coli* and *A. niger*. 1,8-cineole, an aromatic compound found in natural products, exhibits antimicrobial and anti-insect properties and enhances the permeation of transdermal drugs [19,48,71-75]. **Table 3** shows the presence of several minor components, including β-pinene, camphene, α-terpineol, and linalool, across all 3 treatments. A previous study [76] indicated that in addition to 1,8-cineole, other constituents such as carvacrol, γ-terpinene, α-pinene, β-pinene, and limonene also exhibit significant antimicrobial properties, and contribute to synergistic action.

In addition, 1,8-cineole is known to possess antimicrobial properties and is a recognized permeation enhancer [77]. Furthermore, the synergistic effects of both the major and minor components could enhance the overall biological activity of essential oils [78], emphasizing the need for a holistic assessment of the chemical composition concerning bioactivity. Essential oils rich in hydrophobic compounds often involve the integration of these constituents into the microbial cell membrane, leading to permeability alterations, loss of membrane integrity, and ultimately cell death [79,80]. For example, 1,8-cineole has been shown to disrupt bacterial membranes and drastically alter respiration processes, while compounds like carvacrol can inhibit critical metabolic enzymes [81]. Ethyl cinnamate, methyl cinnamate and n-pentadecane were evaluated, and the results proved to have potent antibacterial activity owing to the higher passive permeability of the bacterial cell membrane (*E. coli* O157:H7), followed by crucial intracellular component efflux [17]. Evaluation of the physicochemical and biological activities of *A. galanga* (L.) Willd. essential oils extracted by PEF and ultrasonication pre-treatments are schematically presented in **Figure 1**.

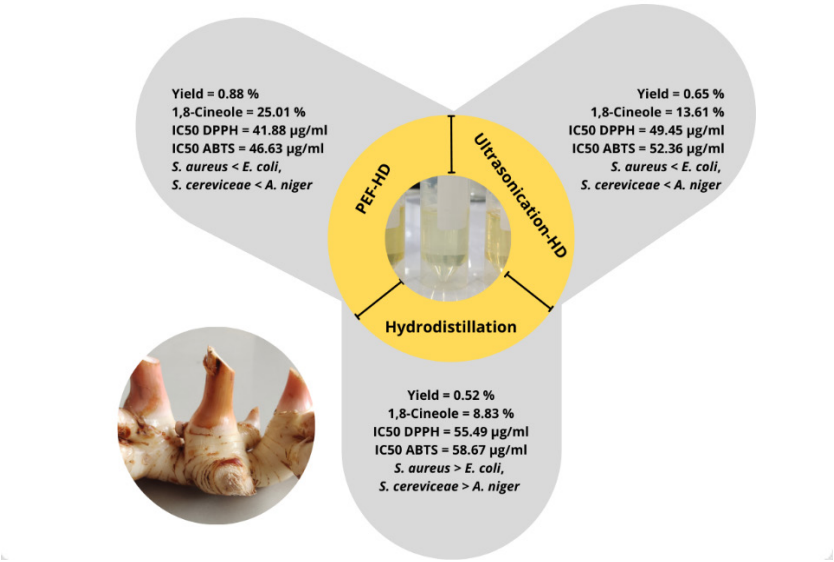

Figure 1 The schematic evaluation of physicochemical and biological activities of *A. galanga* (L.) Willd. essential oil extracted by PEF and ultrasonication pretreatments before hydrodistillation.

Conclusions

This study evaluated the yield, chemical composition, and biological activity of GEO from *A. galanga* (L.) Willd. with different pre-treatments before distillation: PEF and ultrasonication. The average yield of GEO was 0.52 %(w/w) without pre-treatment, 0.88 %(w/w) with PEF pre-treatment, and 0.65 %(w/w) with ultrasonication pre-treatment. The PEF pre-treatment resulted in the highest yield. The highest relative constituent percentage was 25.01% for 1,8-cineole in the PEF pre-treatment, followed by 13.61% in the ultrasonication pre-treatment, and 8.83% in the hydrodistillation control. GEO with PEF pre-treatment also exhibited enhanced antioxidant activity (IC_{50} DPPH: 41.88 µg/mL, ABTS: 46.43 µg/mL), followed by ultrasonication and then hydrodistillation. In antimicrobial tests, GEO with PEF and ultrasonication showed 33% and 14% greater inhibition against *E. coli* than against *S. aureus*. PEF pre-treatment was markedly more effective against *A. niger* than yeast. These results suggest that PEF-treated GEO from *A. galanga* (L.) Willd. has potential in essential oil extraction industry as an antioxidant and antimicrobial agent owing to its increased extraction efficiency, release of bioactive compound and sustainability. Future research should investigate the use of raw materials from various available *Alpinia* origins to explore chemotypic variations, evaluate the feasibility, energy consumption, cost implications of scaling up and conduct stability studies over time.

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Declaration of Generative AI in Scientific Writing

The authors take full responsibility for the content and conclusions of this work. The authors acknowledge the use of generative AI tool (Grammarly) in the preparation of this manuscript, particularly for language editing and grammar correction. No content generation or data interpretation was performed by AI.

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R Amilia Destryana: Conceptualization; Methodology; Formal Analysis; Writing - Original Draft. **Teti Estiasih**: Validation; Supervision; Writing-Review, and Editing. **Sukardi**: Visualization; Investigation; Writing, Review, and Editing. **Dodyk Pranowo**: Data Curation; Validation.

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