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COMPARATIVE BIOACTIVITIES OF ESSENTIAL OILS FROM KAFFIR LIME (*Citrus hystrix*) PEEL WASTES AND BENTONG GINGER (*Zingiber officinale* var. Bentong) RHIZOMES

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Abstract – *Essential oils derived from kaffir lime (Citrus hystrix) peels and Bentong ginger (Zingiber officinale var. Bentong) rhizomes have promising aromatic and bioactive properties, attracting interest for their potential as natural additives to enhance the biological performance and market value of existing products. This study evaluated the phytochemical profiles, antioxidant and anti-inflammatory activities of essential oils extracted from kaffir lime peels and ginger rhizomes using ultrasonication-assisted hydrodistillation. Gas Chromatography-Mass Spectrometry revealed β-pinene (19.9%) and citronellal (18.7%) as major compounds in kaffir lime peel essential oil. In comparison, Bentong ginger rhizome essential oil predominantly contained α-citral (22%) and α-curcumin (15.5%). Bentong ginger rhizome essential oil exhibited higher total phenolic (1.78±0.14 mg GAE/100 µL EO) and flavonoid (3.37±0.04 mg QE/100 µL EO) contents, correlating with superior antioxidant activities in ferric reducing power (34.07±1.54 µmol Fe^{2+}/g of EO) and radical scavenging (DPPH: 77.55±1.57% & ABTS: 67.58±1.34% at 50 mg/mL) effects compared with kaffir lime peel essential oil at similar concentrations tested. Kaffir lime peel essential oil showed stronger ferrous ion chelating activity (82.17±1.42% at 0.8 mg/mL) besides exerting superior anti-inflammatory properties reflected by higher inhibitory effect (70.69±0.22% at 10 mg/mL) towards bovine serum albumin denaturation as compared to Bentong ginger rhizome essential oil at the same concentration. β-pinene, a major compound of kaffir lime peel essential oil, had been recognized for its metal chelating and anti-inflammatory effects in previous studies. Kaffir lime peel offer a more cost-effective and eco-friendlier raw material to produce essential oil with competitive bioactivity compared to the high-priced Bentong ginger.*

Keywords: antioxidant, anti-inflammatory, Bentong ginger rhizomes, essential oils, kaffir lime peels.

I. INTRODUCTION

Citrus hystrix, or kaffir lime, is native to Southeast Asia and belongs to the Rutaceae family. The fruit, typically small and round, is highly aromatic and plays a crucial role as a spice in the preparation of tomyam, a well-known dish in Malaysia and Thailand. Its peels, often discarded as waste, are rich in phenolics and flavonoids with proven antioxidant and anti-inflammatory activities [1]. The study by Lubinska-Szczygeł et al. [1] demonstrated that the antioxidative content in kaffir lime peel was significantly higher than in the pulp. *Zingiber officinale* var. Bentong, commonly referred to as Bentong ginger, is a highly regarded variety of ginger from the town of Bentong in Pahang, a key ginger cultivation area in Malaysia. This herbaceous perennial plant from the Zingiberaceae family is the most expensive ginger variety in Malaysia, prized for its warm flavour, large rhizomes, and premium antioxidant properties linked to its distinct phenolic profile [2]. This high-value ginger variety is increasingly sought after for natural health applications.

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The increasing consumer preference for eco-friendly and natural health products like essential oils (EOs) from aromatic plants underscores the need to explore novel sources like kaffir lime peel and Bentong ginger rhizome, which present a pleasant and universally acceptable aroma. EOs are well-regarded for their bioactivities and are clinically proven to possess positive physiological effects on the central nervous system with concomitant psychological benefits, thus promoting mental health and strengthening immunity [3]. While traditional EO markets are dominated by mint, orange, lemon, and lavender, there are issues pertaining to their adverse reactions and limited applications. All these factors highlight the importance of expanding EO diversity [4, 5]. This study aims to compare the phytochemical profiles and bioactivities of EOs from the two aforementioned Malaysian plants, contributing to the exploration of high-performance EOs for diverse commercial applications.

II. LITERATURE REVIEW

The EO extracted from kaffir lime peels usually contains key bioactive compounds, including sabinene, pinene, citral, citronellal, limonene, linalool, and terpinen-4-ol, which contribute to its proven antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, and mosquito-repellent properties [6, 7]. These health benefits make kaffir lime peel a promising resource for the trash-to-treasure market, transforming what is often considered food or agro-industrial waste into valuable products. It is estimated that 40–60% of the total mass of citrus fruits results in peel waste [8]. As such, the repurposing of kaffir lime peel is gaining momentum, with its potential applications in the production of natural cleaners, extracts, and supplements. This not only adds economic value but also contributes to sustainable development by reducing the burden of food waste. Moreover, the EOs of ginger have also demonstrated antimicrobial, anti-inflammatory, anticancer, anodyne, and cell-protective activities [9, 10]. Major volatile secondary metabolites extracted from the ginger rhizomes include zingiberene, citral, nerolidol, curcumene, bisabolene, and farnesene, which contribute to Bentong ginger's potential to produce a wide range of commodities, such as food preservatives, medications, perfumes, and cosmetics [11]. Although various bioactivities of kaffir lime peel EO (KEO) and Bentong ginger rhizome EO (BEO) have been studied in different research, a more in-depth comparative analysis of these EOs under identical conditions is limited, thus hindering the comprehensive assessment of their bioactivities [1, 11]. Because EOs are complex mixtures of volatile chemical compounds from plants, their compositions can vary significantly depending on factors such as the plant's geographic origin, harvesting period, post-harvest conditions, and extraction methods [12]. Hence, through a standardized extraction approach, the primary objective of this research aimed to compare the antioxidant and anti-inflammatory properties of EOs of fresh kaffir lime peels and Bentong ginger rhizomes using various chemical assays and relating the EOs' compositions to their bioactivities. This would provide more explicit guidance for their future processing and use in downstream applications.

III. RESEARCH METHODS

A. Sample collection and preparation

Kaffir limes and Bentong gingers were collected from a farm in Jalan Semenanjung, Sabak, Selangor, and from Village Home Grocer in Kampung Bukit Tinggi, Bentong, Pahang, respectively. A total of four kilograms each of fresh kaffir lime fruits and Bentong ginger rhizomes were purchased and stored at –20°C before processing. Both were washed thoroughly with tap water to remove surface impurities. For kaffir limes, damaged and rotten fruits were discarded, and cut clean peels, about 1 mm thick, were obtained by using a grater to peel off, yielding around 1 kg of peel sample from four kilograms of fruits. For Bentong gingers, contaminated and darkening parts were removed, and the rhizomes were cut into approximately 5 mm thick pieces using a knife, mortar, and pestle. Both prepared

samples were then packed in plastic bags and stored in a –20°C freezer for preservation prior to extraction.

B. Extraction method and physical property characterization of EOs

The ultrasonication-assisted hydrodistillation (UAE-hydrodistillation) protocol for EOs was adapted from da Silva Moura et al. [13] with modifications. EO extraction began with a sonication pre-treatment, where the cut kaffir lime peels or Bentong ginger rhizomes were mixed with Milli-Q water in a 500 mL round-bottom flask. The flask was then sonicated for 30 minutes using an ultrasonic bath (Elmasonic P Series). After adding anti-bumping granules, the mixture was subjected to hydrodistillation at approximately 100^{o}C until EO extraction stabilised. The distillate was transferred to a separatory funnel to isolate the EOs from the aqueous layer, with further water rinsing to ensure complete oil recovery. Anhydrous sodium sulphate was added to remove excess water, and the purified EOs were stored in a universal bottle, sealed with parafilm and aluminium foil, and kept at 4^{o}C before further analysis. The colour and odor of EOs were recorded. This process was repeated several times to accurately determine the EO yield for each plant material according to Formula (1).

$$\%\text{Yield} = \frac{\text{Weight of EO obtained (g)}}{\text{Weight of plant material used (g)}} \times 100\% \quad (1)$$

C. Characterization of volatile compounds in EOs

The Agilent Technologies 7890A Gas Chromatography–Mass Spectrometry (GC-MS) system with a DB-WAX UI column (30 m x 0.25 mm x 0.25 μm) was used to identify volatile phytochemicals in the extracted EOs. The inlet temperature was set at 250^{o}C, and the analysis was performed in split mode at a 15:1 ratio, with a 1 μL sample injection. Helium served as the carrier gas, with a flow rate of 0.9 mL/min. For KEO, a modified temperature program based on Lubinska-Szczygeł et al. [6] was used. The oven temperature started at 40^{o}C, held for two minutes, then increased to 90^{o}C at 5^{o}C/min, followed by a ramp to 220^{o}C at 30^{o}C/min, with no hold, and held for seven minutes, for a total runtime of approximately 23 minutes. For BEO, the temperature program, adapted with modifications from Liang et al. [14], began at 40^{o}C, held for three minutes, then increased to 60^{o}C at 10^{o}C/min, followed by a rise to 90^{o}C at 5^{o}C/min, then an immediate ramp to 240^{o}C at 4^{o}C/min, held for eight minutes, with a total runtime of approximately 57 minutes. Prior to GC-MS analysis, the EO sample was diluted with GC-MS grade n-hexane (Merck) to achieve a concentration of 20 μL/mL. The diluted solution was filtered into a GC-MS vial using a 0.22 μm Nylon membrane filter. After filtration, the EO solution was injected into the GC-MS for chromatogram acquisition, with peak identification achieved by comparing retention times and their respective mass spectra with the mass spectral reference library from National Institute of Standards and Technology (NIST) [15]. The peak areas were used to quantify the percentage of each identified component presented in the sample.

D. Determination of total phenolic content (TPC) in EOs

The TPC assay protocol was adapted from Sari et al. [16] with modifications. In a 15 mL centrifuge tube, 100 μL of the methanol-diluted EO (1:50 dilution) or gallic acid standard (prepared from a 2.5 mg/mL stock in methanol) was mixed with 3.8 mL distilled water and 100 μL Folin–Ciocalteu's phenol reagent. The mixture was vortexed and left to stand for five minutes. Subsequently, 1 mL of 10% sodium carbonate was added, the mixture was vortexed again, and the reaction volume was adjusted to 5 mL. The reaction mixture was incubated in the dark at room temperature for two hours, and absorbance was measured at 750 nm using a UV–Vis spectrophotometer. A blank was prepared by replacing the sample or standard with methanol. TPC was expressed as mg gallic acid equivalents (GAE) per 100 μL of EO based on a

gallic acid standard curve (0.1–1.35 mg/mL). All procedures were performed in triplicate. The TPC value is calculated based on Formula (2), where the Abs. is the absorbance of the tested sample at 750 nm, while d = dilution factor for the tested sample, b = y-intercept, and m = slope of the linear equation of gallic acid standard curve on Formula (2).

$$\text{TPC} = \frac{\frac{\text{Abs.} - \text{b}}{\text{m}} \times \text{d}}{10} \quad (2)$$

E. Determination of total flavonoid content (TFC) in EOs

The TFC assay protocol was adapted from Shraim et al. [17] with modifications. In a 15 mL centrifuge tube, 500 μL of the methanol-diluted EO (1:40 dilution) or quercetin standard (prepared from a 2.5 mg/mL stock in methanol) was mixed with 2 mL methanol and 150 μL of 1M sodium nitrite solution. After vortex, the mixture was left to stand for three minutes. Next, 150 μL of 10% aluminium chloride solution was added, vortexed, and left for another three minutes. Then, 1 mL of 1M NaOH and 1.2 mL methanol were sequentially added with vortex mixing to bring the final volume to 5 mL. The reaction mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 415 nm using a UV–Vis spectrophotometer. A blank was prepared by replacing the sample or standard with methanol. TFC was expressed as mg quercetin equivalents (QE) per 100 μL of EO based on a quercetin standard curve (0.25–1.5 mg/mL). All procedures were performed in triplicate. The TFC value is calculated based on Formula (3), where the Abs. is the absorbance of the tested sample at 415 nm, while d = dilution factor for the tested sample, b = y-intercept and m = slope of the linear equation of quercetin standard curve on Formula (3).

$$\text{TFC} = \frac{\frac{\text{Abs.} - \text{b}}{\text{m}} \times \text{d}}{10} \quad (3)$$

F. Ferric reducing antioxidant power (FRAP) assay

A fresh FRAP reagent was prepared by mixing 20 mM ferric chloride, 10 mM TPTZ in 40 mM HCl, and 300 mM acetate buffer (pH 3.6) in a 1:1:10 volume ratio. In a 96-well microplate, 10 μL of EO solution (10 mg/mL diluted from a 50 mg/mL stock in methanol) or $FeSO_4$ standard solution (prepared from a 1 mM stock in distilled water) was mixed with 190 μL of the FRAP reagent. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 593 nm using a microplate reader [18]. The same steps were performed by replacing the 10 μL EO sample with ascorbic acid to form the positive control mixture. A blank was prepared by replacing the EOs with methanol while the standard and positive control with distilled water. FRAP value was expressed as μmol Fe^{2+} equivalents per gram of EO based on a $FeSO_4$ standard curve (0.1–0.6 mM). All procedures were performed in triplicate. The FRAP value is calculated based on Formula (4), where the Abs. S is the absorbance of the sample (EOs or positive control), Abs. B is the absorbance of the blank, Abs. D is the decoloration absorbance applied only when calculating the FRAP value of the EO sample, and C is the sample concentration (in g/μL) used, while b and m represent the y-intercept and the slope of the linear equation of $FeSO_4$ standard curve, respectively on Formula (4).

$$\text{FRAP value} = \frac{(\text{Abs. S} - \text{Abs. B} - \text{Abs. D}) - \text{b}}{\text{m} \times \text{C} \times 1000} \quad (4)$$

G. Metal chelating assay

In a 96-well microplate, 50 μL of EO sample (0.05–0.8 mg/mL prepared from a 50 mg/mL stock in methanol) was mixed with 20 μL of 0.5 mM ferrous chloride solution and 160 μL methanol, then left to stand for five minutes. Next, 20 μL of 2.5 mM ferrozine solution was added, and the reaction mixture was incubated in the dark at room temperature for 10 minutes.

Absorbance was measured at 562 nm using a microplate reader [19]. For the EDTA (positive control) reaction mixture, the same steps were performed by replacing the 50 μL EO sample with EDTA solution (0.05–0.8 mg/mL prepared from a 1 mg/mL stock in distilled water). Methanol or distilled water was used to replace the 50 μL of EO sample or EDTA solution, respectively, to form the control mixtures. All procedures were performed in triplicate. The ferrous ion chelating activity of the tested EOs or EDTA solutions is calculated based on Formula (5), where the Abs. C is the absorbance of the control mixture, Abs. S is the absorbance of the sample (EOs or positive control), while the decoloration absorbance, Abs. D is only applied when calculating the chelating activity of the EO sample.

$$\text{Metal chelating activity} = \frac{\text{Abs. C} - (\text{Abs. S} - \text{Abs. D})}{\text{Abs. C}} \times 100\% \quad (5)$$

H. DPPH radical scavenging assay

In a 96-well microplate, 20 μL of EO sample (1–50 mg/mL prepared from a 50 mg/mL stock in methanol) was mixed with 180 μL of 200 μM DPPH solution (freshly prepared from a 600 μM stock in methanol, stored at –20^{o}C). The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 515 nm using a microplate reader [20]. For the ascorbic acid (positive control) reaction mixture, the same steps were performed by replacing the 20 μL of EO sample with an ascorbic acid solution (1–50 mg/mL prepared from a 100 mg/mL stock in distilled water). Methanol or distilled water was used to replace the 20 μL of EO sample or ascorbic acid solution, respectively, to form the control mixtures. All procedures were performed in triplicate. The DPPH radical scavenging activity of the tested EOs or ascorbic acid solutions is calculated based on Formula (6), where the Abs. C is the absorbance of the control mixture, Abs. S is the absorbance of the sample (EOs or positive control), while the decoloration absorbance, Abs. D is only applied when calculating the scavenging activity of the EO sample:

$$\text{DPPH scavenging activity} = \frac{\text{Abs. C} - (\text{Abs. S} - \text{Abs. D})}{\text{Abs. C}} \times 100\% \quad (6)$$

I. ABTS radical scavenging assay

In a 96-well microplate, 10 μL of EO sample (1–50 mg/mL prepared from a 50 mg/mL stock in methanol) was mixed with 195 μL of ABTS reagent. The reaction mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 734 nm using a microplate reader [21]. For the ascorbic acid (positive control) reaction mixture, the same steps were performed by replacing 10 μL of EO sample with an ascorbic acid solution (1–50 mg/mL prepared from a 100 mg/mL stock in distilled water). To form the control mixtures, methanol or distilled water was used to replace the 10 μL of EO sample or ascorbic acid solution. All procedures were performed in triplicate. The ABTS radical scavenging activity of the tested EOs or ascorbic acid solutions is calculated based on Formula (7), where the Abs. C is the absorbance of the control mixture, Abs. S is the absorbance of the sample (EOs or positive control), while the decoloration absorbance, Abs. D is only applied when calculating the scavenging activity of the EO sample.

$$\text{ABTS scavenging activity} = \frac{\text{Abs. C} - (\text{Abs. S} - \text{Abs. D})}{\text{Abs. C}} \times 100\% \quad (7)$$

J. Bovine serum albumin (BSA) denaturation inhibition assay

In a 15 mL centrifuge tube, 50 μL of EO sample (0.5–10 mg/mL prepared from a 50 mg/mL stock in methanol) was mixed with 450 μL of 5% BSA buffered solution (prepared by dissolving 1 g BSA powder in 20 mL PBS at pH 6.3). The mixture was incubated in a water bath at 37°C for 20 minutes, followed by heating at 70°C for five minutes. Next, 2.5 mL of PBS (pH 6.3) was added to achieve a final reaction volume of 3 mL, and the mixture was vortexed. Turbidity absorbance was measured at 416 nm using a UV-Vis spectrophotometer [22]. Distilled water was used

as a blank. For the Diclofenac sodium (positive control) reaction mixture, the same steps were performed by replacing the 50 μL of EO sample with Diclofenac sodium solution (0.5–10 mg/mL prepared from a 50 mg/mL stock in methanol). Methanol was used to replace the 50 μL of EO sample or Diclofenac sodium solution to form the control mixture. All procedures were performed in triplicate. The anti-denaturation capacity for BSA of the tested EOs or Diclofenac sodium solutions is calculated based on Formula (8), where the Abs. C is the absorbance of the control mixture and Abs. S is the absorbance of the sample (EOs or positive control).

$$\text{Anti-BSA denaturation capacity} = \frac{\text{Abs. C} - \text{Abs. S}}{\text{Abs. C}} \times 100\% \quad (8)$$

K. Statistical data analysis

Data analysis was performed using SPSS software (version 27). All experiments were conducted in triplicate and were presented as the mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

IV. RESULTS AND DISCUSSION

A. Yield and characteristic of EOs Extracted

According to Table 1, the extraction yield of KEO was significantly higher than that of BEO. The yield of KEO was about two-fold higher than the 0.8% (v/w) reported by Houng et al. [8], who employed traditional hydrodistillation with extraction material and duration consistent with those used in this research. This suggested that the use of UAE-hydrodistillation in this study may have contributed to the higher yield, as the non-thermal ultrasound pretreatment was shown to have a duration-dependent impact on the yield of kaffir lime leaf EO, as reported by Mohideen et al. [23]. Ultrasonication enhances oil extraction by causing cavitation bubbles to implode, which leads to cell wall separation, thereby facilitating rapid exudation and water penetration during hydrodistillation. This process helps preserve thermolabile and volatile compounds, minimising heat degradation. As the pretreatment duration increases, the average oil yield tends to rise. However, controlling ultrasound treatment time is crucial, as excessive duration can increase temperatures and cause volatile components to vaporise before extraction, thus reducing yield. Mohideen et al. [23] identified the optimal ultrasound pretreatment time as two hours for maximum efficiency on the yield of kaffir lime leaf EO. In this study, a shorter ultrasonication time of 30 minutes was used to prevent potential oil loss due to overexposure to ultrasonic energy during the pretreatment. However, the ultrasonication pretreatment had a less significant impact on increasing the extraction yield of the BEO studied, with the obtained yield of 0.104 $\pm$ 0.040% (w/w) based on Table 1. This was notably lower than the yield range of 0.29–0.43% (v/w) reported by Jahiddin [24], who used normal hydrodistillation to extract EO from oven-dried Bentong ginger rhizomes. The lower yield observed for BEO in this study may be attributed to the high moisture content within the fresh plant material used due to the absence of drying pretreatment. While drying significantly boosts EO yield, it also alters the aroma profile, intensifying the spicy and earthy notes due to the higher extraction of sesquiterpenes, while reducing the lighter, fresher, and lemony monoterpenes typically found in fresh ginger EOs as the major content [25]. This shift in aroma may be less appealing for industries such as perfumery, cosmetics, and aromatherapy, where a pleasant fragrance is crucial for customer satisfaction. The odour of the KEO was characterised as citrus, minty, herbal, and woody scent similarly described by Lubinska-Szczygeł et al. [6]. In comparison, the sensory analysis by Liang et al. [14] identified citrus-like and spicy attributes as the key orthonasal and retronasal olfaction of unpeeled ginger extracts respectively, which aligned with the odour description of BEO in the present study.

Table 1: Extraction yield of EOs and their physical property by UAE-hydrodistillation

Parameter	KEO	BEO
Yield (%, w/w)	1.629 ± 0.240^{a}	0.104 ± 0.040^{b}
Colour	Pale yellow	Orange brown
Odour	Strong citrusy and uplifting aroma with some hint of floral and sweet smelling accompanied by a slightly aftertaste tart	Energising warm, woody, and sharp with a touch of sweetness, while having a slightly peppery undertone with a subtle lemony hint

Note: Yield percentages are expressed as Mean ± S.D. ($n = 6$). Means sharing same superscript letter are not significantly different ($p < 0.05$)

B. Phytochemicals of KEO through GC-MS analysis

Studies by Lubinska-Szczygeł et al. [6] and Katerina et al. [7] indicated that EOs extracted from kaffir lime peel typically contained β-pinene, limonene, and sabinene as the major compounds. A common finding in both studies was that the content of β-pinene and monoterpenes was generally higher than that of α-pinene and sesquiterpenes. This observation was consistent with the phytochemical profile of KEO determined by the GC-MS analysis in this research, as shown in Figure 1. Based on the results presented in Table 2, a total of fifteen compounds were identified in the KEO, comprising 95.29% monoterpenes and 4.71% sesquiterpenes. Regarding functional group distribution, 48.92% were oxygenated terpenoids, while 51.08% were hydrocarbon terpenes. The five most abundant terpenes and terpenoids were β-pinene (19.86%), citronellal (18.65%), citronellol (11.55%), sabinene (11.33%), and limonene (10.56%), all of which contributed to the herbal, citrus, and woody aroma of the KEO. These compounds also demonstrated bioactive properties, with β-pinene and sabinene particularly noted for their antioxidative and anti-inflammatory effects [26–29]. Other minor compounds identified included terpinen-4-ol, α-terpineol, and linalool, all of which belong to the oxygenated monoterpene group. The findings were consistent with those of Sreepian et al. [30], where a slight predominance of β-pinene over limonene was observed. It should be noted that terpene content in KEO may vary depending on factors such as geographical origin, fruit ripeness, and the extraction method [12].

C. Phytochemicals of BEO through GC-MS analysis

According to Munda et al. [11], ginger rhizome EOs typically contained curcumene, geranial (α-citral), neral (β-citral), camphene, zingiberene, and bisabolene as the major compounds. Additionally, their study noted that the sesquiterpene content in ginger rhizome EOs was generally higher than that of monoterpenes, as revealed by GC-MS analysis, which contrasted with the monoterpene-dominant profile of KEO mentioned earlier. But interestingly, the phytochemical profile of BEO determined in this study was in discordance with the literature (see Figure 2). Based on the results presented in Table 3, thirteen compounds were identified in the BEO, comprising 54.62% monoterpenes and 45.38% sesquiterpenes. This relatively balanced composition of monoterpenes and sesquiterpenes in the BEO differed from many previous studies, which generally found ginger rhizome EOs to be sesquiterpene-dominant, as mentioned earlier. However, one study by Gupta et al. [31] reported a monoterpene-rich ginger EO from ginger sourced from Nahan, India. In terms of functional groups, the BEO contained 54.22% oxygenated terpenoids and 45.78% hydrocarbon terpenes. This contrasted with the terpene profile of KEO, where hydrocarbon terpenes were slightly more abundant than oxygenated terpenoids. Furthermore, according to Sasidharan et al. [25], EOs extracted from fresh ginger typically contained more oxygenated compounds than dried ginger. Thus, a higher oxygenated terpenoid composition was expected for the BEO in this study. The three most abundant terpenes and terpenoids in the BEO were α-citral (21.96%), α-curcumene (15.48%), and β-citral (12.96%), which contributed significantly to its warm and lemony aroma. The predominance of α-citral over its beta isomer was also found in the EOs extracted from

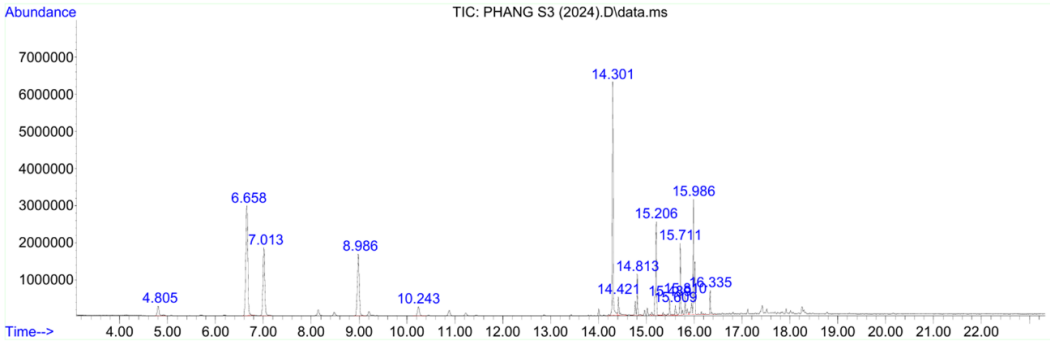

Fig. 1: GC-MS chromatogram of KEO

Table 2: Volatile phytochemicals detected by GC-MS for KEO

No	T_R (min)	Compound	Molecular Formula	Chemical Family	Composition (%)	Quality*
1	4.805	α-Pinene	$C_{10}H_{16}$	Monoterpenes	1.564	97
2	6.658	β-Pinene	$C_{10}H_{16}$	Monoterpenes	19.857	94
3	7.013	Sabinene	$C_{10}H_{16}$	Monoterpenes	11.334	91
4	8.986	D-Limonene	$C_{10}H_{16}$	Monoterpenes	10.558	95
5	10.243	γ-Terpinene	$C_{10}H_{16}$	Monoterpenes	1.556	96
6	14.301	(R)-(+)-Citronellal	$C_{10}H_{18}O$	Aldehyde, Monoterpenoid	18.650	97
7	14.421	Copaene	$C_{15}H_{24}$	Sesquiterpenes	1.888	97
8	14.813	Linalool	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	3.842	97
9	15.206	(-)-Terpinen-4-ol	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	7.907	97
10	15.489	2,6-Dimethyl 2,6-octadiene	$C_{10}H_{18}$	Monoterpenes	1.508	97
11	15.609	α-Caryophyllene	$C_{15}H_{24}$	Sesquiterpenes	0.889	98
12	15.711	α-Terpineol	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	4.913	86
13	15.810	Germacrene D	$C_{15}H_{24}$	Sesquiterpenes	1.930	99
14	15.986	(R)-(+)-β-Citronellol	$C_{10}H_{20}O$	Alcohols, Monoterpenoids	11.552	96
15	16.335	*trans*-Geraniol	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	2.051	97

*Note: Only compounds with a quality of more than 85% are considered**

the ginger rhizomes sourced from Costa Rica and India through hydrodistillation, according to the GC-MS analysis from González-Guevara et al. [9] and Singh et al. [10], respectively. Several of these major terpenes have been shown to possess favourable bioactive properties in various studies. For instance, citral has been proven to have significant antioxidant activity, as demonstrated by Baschieri et al. [32], who reported that it slowed the autoxidation of standardised substrates by generating oxygenated radicals that terminate both their own reactions and the oxidative chain of the substrate rapidly. In addition to the major compounds, minor components such as β-bisabolene, trans-geraniol, γ-cadinene, α-farnesene, and camphene were also identified in the BEO. Notably, trans-geraniol was the only compound found in both the KEO and BEO. This monoterpenoid, commonly used in perfumery, not only contributed to the pleasant floral scent of both the EOs studied but also exhibited promising antioxidative and anti-inflammatory activities by

effectively scavenging DPPH radicals and inhibiting the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), as reported by Chen et al. [33].

D. TPC and TFC detected in EOs

TPC represents the concentration of phenolic compounds in a plant extract, which are often associated with antioxidant and anti-inflammatory properties. Phenolic compounds are secondary metabolites in plants that can donate hydrogen atoms or electrons, effectively neutralise ROS and prevent oxidative damage, thereby reducing the activation of pro-inflammatory pathways [34]. In this study (see Table 4), the TPC of BEO was significantly higher than that of KEO, based on the gallic acid calibration curve obtained ($y = 1.6823x + 0.1399$, $R^2 = 0.99$). In separate studies, Lubinska-Szczygeł et al. [6] and Osae et al. [35] determined the TPC values (expressed as mg GAE/g of extract) of 22.6±2.1 and 133.4±1.9 for EOs from fresh kaffir lime peels of Thai origin and fresh ginger rhizomes of Ghanaian origin, respectively. Both studies applied a similar extraction approach to obtain the EOs. Thus, the comparative results of this study further supported the typically higher TPC found in BEO compared to KEO, despite geographical differences in the plant sources.

TFC refers to the concentration of flavonoids, another class of phytochemicals known for their antioxidant and anti-inflammatory effects. They have been demonstrated to inhibit the release of pro-inflammatory mediators by blocking key signalling pathways involved in inflammation, such as NF-kB and MAPKs [36]. According to Table 4, the TFC of BEO was more than double that of KEO, based on the quercetin calibration curve obtained ($y = 1.426x + 0.2478$, $R^2 = 0.99$). However, this outcome was in discordance with the literature, where Mohideen et al. [37] measured a higher TFC value (expressed as mg QE/g of extract) of 145.8 for the EO from kaffir lime peels of Malaysian origin than the 0.074 evaluated by Deleanu et al. [38] for the ginger rhizome EO of Chinese origin. The different TFC values could be due to the variation in the plant origins and extraction conditions between the two studies and ours. Hence, the findings of this research potentially proved that under standardised conditions, BEO could have a higher TFC than that of KEO. Promising biological activities were expected for the KEO and BEO extracted due to high TPC and TFC values. Many studies have demonstrated a strong correlation between various natural extracts' antioxidant activity and phenolic content [36, 39].

E. FRAP and metal chelating activity of EOs

One key mechanism by which antioxidants protect cells and tissues from oxidative damage is donating electrons, which neutralises free radicals or reduces oxidised molecules [40]. In this study, the FRAP assay was based on the ability of antioxidants in the EOs extracted to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). As shown in Figure 3, the FRAP value of BEO (34.07±1.54) was approximately two-fold higher than the KEO (17.84±1.13), by referring to the $FeSO_4$ standard curve ($y = 0.7781x - 0.0318$, $R^2 = 0.9971$) obtained. This agreed with the literature, where Ivanović et al. [18] determined a higher FRAP value (expressed μmol Fe^{2+}/ml of EO) of 10.74 for ginger rhizome EO of Malagasy origin than the 0.004 reported by Saeio et al. [41] for kaffir lime EO of Thai origin. Both studies used the Clevenger apparatus to extract the EOs by hydrodistillation for three hours.

The distinctly lower FRAP value of the Thai kaffir lime EO may be because kaffir lime leaves were used instead of peels to obtain the EO [41]. The higher TPC and TFC of BEO could contribute to its stronger FRAP performance, as these phenolic compounds with multiple electron-withdrawing and electron-donating substituents, such as hydroxyl groups (–OH), significantly influence the antioxidant capacity of plant extracts [34]. Citrals (both α-citral and β-citral), the most abundant monoterpenoids found in the BEO studied are also the main compounds believed to be responsible for the antioxidant properties of BEO.

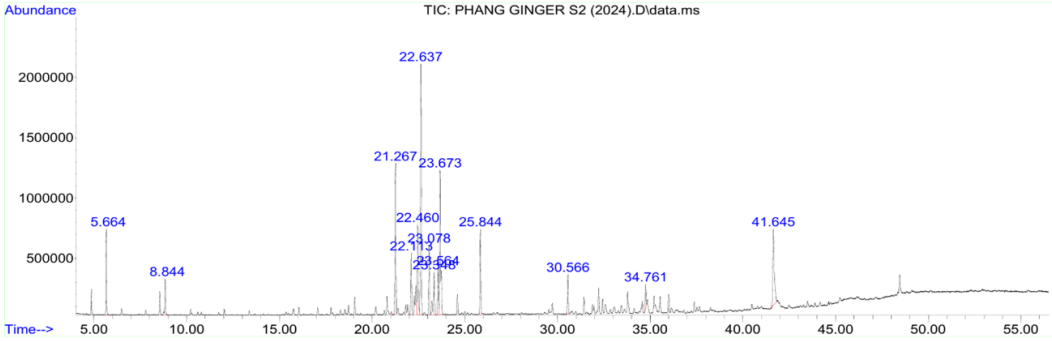

Fig. 2: GC-MS chromatogram of BEO

Table 3: Volatile phytochemicals detected by GC-MS for BEO

No	T_R (min)	Compound	Molecular Formula	Chemical Family	Composition (%)	Quality*
1	5.664	Camphene	$C_{10}H_{16}$	Monoterpenes	5.594	96
2	8.844	Eucalyptol	$C_{10}H_{18}O$	Ethers, Monoterpenoids	2.883	98
3	21.267	β-Citral	$C_{10}H_{16}O$	Aldehydes, Monoterpenoids	12.957	95
4	22.113	γ-Cadinene	$C_{15}H_{24}$	Sesquiterpenes	5.930	96
5	22.460	β-Bisabolene	$C_{15}H_{24}$	Sesquiterpenes	8.910	95
6	22.637	α-Citral	$C_{10}H_{16}O$	Aldehydes, Monoterpenoids	21.955	94
7	23.078	α-Farnesene	$C_{15}H_{24}$	Sesquiterpenes	5.713	97
8	23.348	*cis*-Geraniol	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	3.961	86
9	23.564	β-Sesquiphellandrene	$C_{15}H_{24}$	Sesquiterpenes	4.159	96
10	23.673	α-Curcumene	$C_{15}H_{22}$	Sesquiterpenes	15.478	99
11	25.844	*trans*-Geraniol	$C_{10}H_{18}O$	Alcohols, Monoterpenoids	7.271	97
12	30.566	Nerolidol	$C_{15}H_{26}O$	Alcohols, Sesquiterpenoids	3.301	91
13	34.761	β-Eudesmol	$C_{15}H_{26}O$	Alcohols, Sesquiterpenoids	1.887	99

*Note: Only compounds with a quality of more than 85% are considered**

Table 4: Phenolic contents determined in the EOs extracted

Parameter	KEO	BEO
TPC (mg GAE/100 μL EO)	0.86 ± 0.05^a	1.78 ± 0.14^b
TFC (mg QE/100 μL EO)	1.36 ± 0.02^a	3.37 ± 0.04^b

Note: Means ± S.D. (n = 3) present in the same row with different superscript letter are not significantly similar ($p < 0.05$)

Based on the study by Ling et al. [42], high antioxidative activities of EOs from two citral-rich Cinnamomum spp. were reported. A significant proportional relationship was found between the FRAP values and the citral contents.

Antioxidants with strong reducing power, indicated by high FRAP values, can paradoxically catalyse ROS production via Fenton reactions involving transition metals like Fe^{2+} produced by the reduction of Fe^{3+}, generated by antioxidants. This ROS generation contributes to cellular oxidative damage and aging. Therefore, comprehensive antioxidant evaluation requires assays be-

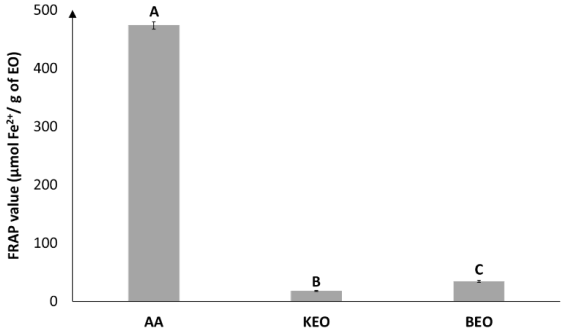

Fig. 3: Effects of KEO and BEO on the reduction of Fe^{3+} as measured in FRAP assay Ascorbic acid acts as the positive control.

Note: Bars with different alphabets are not significantly similar ($p < 0.05$)

yond FRAP, such as metal chelation assay, which targets ROS production upstream by sequestering metal ions involved in Fenton chemistry [34]. In this research, the metal chelating assay was based on the ability of antioxidants presented in the EOs studied to neutralise (binding to) the ferrous ions. This neutralisation occurs when the EOs bind to the ferrous ions, thus causing decoloration due to the reduction of the coloured ferrous-Ferrozine complex [19]. This mechanism mimics the chelating event of antioxidants to the ferrous ions in biological systems. By binding to these dissociated metals, antioxidants can inhibit their ability to catalyse ROS formation, thereby reducing oxidative stress.

As shown in Figure 4, the ferrous ion chelating percentages of KEO and BEO reached 82.17±1.42% and 63.29±3.75% at 0.8 mg/mL, respectively. The chelating activity observed for the KEO was higher than that of the seven Citrus spp. EOs analysed by Wang et al. [39], which showed metal chelating activities ranging approximately from 5% to 38% at a concentration of 1 mg/mL. In the same study, the ginger EO sourced from India exhibited a chelating activity of 2.43 ± 0.2% at 1 mg/mL, also lower than the chelating percentage recorded for the BEO at 0.8 mg/mL. Thus, the KEO and BEO demonstrated higher metal chelating properties than those reported in the literature, even at lower EO concentrations.

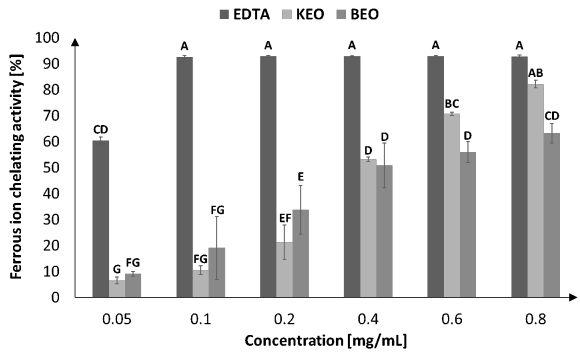

Fig. 4: Metal chelating activities of KEO and BEO. The positive control is EDTA.

Note: Bars with different alphabets are not significantly similar ($p < 0.05$)

Overall, both the EOs exhibited a dose-dependent increase in chelation, with the KEO demonstrating stronger antioxidant potential than the BEO by showing significantly higher metal chelating percentages at concentrations of 0.6 and 0.8 mg/mL. However, at lower concentrations (0.05–0.4 mg/mL), the chelating activity differences between the two EOs were less pronounced. By observing the concentration-dependent trend in Figure 4, it was possible to infer the IC_{50} values for both the EOs. KEO was expected to record a lower IC_{50} due to its faster achievement of 50% chelating activity than the BEO, indicating its stronger chelating capacity. According to Dhanik et al. [43], the metal-chelating IC_{50} of ginger rhizome EO from northern India was estimated to be 25±0.08 mg/mL. Although there was limited work conducted on the metal chelating efficacy of KEO, research by Irawaty et al. [44] provided relevant data with ferrous ion chelating IC_{50} values between 0.12 and 18.50 mg/mL, observed across three different fractions of the kaffir lime peel solvent extracts. Therefore, the present study's findings further supported that the KEO could be a stronger antioxidant than the BEO in terms of metal chelation, likely achieving a lower IC_{50} value.

The stronger chelating performance of KEO could be attributed to its distinct terpene composition compared to that of BEO. Many of the monoterpenes presented in the KEO, such as α-pinene, terpinen-4-ol, linalool, γ-terpinene, and citronellal, had previously been shown to possess higher ferrous ion chelating activity than compounds like citral and eucalyptol, according to a comparative study by Wojtunik-Kulesza et al. [45]. Notably, citral and eucalyptol were identified as major and minor components in the BEO analysed in this study, which may have contributed to its relatively lower chelating activity. Furthermore, β-pinene, the most abundant monoterpene in the KEO, had been shown to exhibit effective cuprous ion chelating properties, as reported by Van Zyl et al. [27]. This specific monoterpene, alongside other highly chelating compounds in the KEO, likely contributed to its superior metal chelating activity compared to the BEO in this research. Although higher TPC and TFC were reported for BEO, which were typically correlated to a stronger antioxidative capacity, as discussed previously, these findings suggested that the terpene profile of KEO may play a key role in enhancing its potential as a natural metal chelator and antioxidant.

F. DPPH and ABTS radical scavenging activities of EOs

While the FRAP and metal chelating assays tested the abilities of the EOs studied to prevent the formation of harmful free radicals, the radical scavenging assays demonstrated the efficiency of the EOs to stabilise and deactivate these unpaired-electrons and reactive radicals via donation of electron or hydrogen atom [40]. In DPPH and ABTS radical scavenging assays, these radicals were scavenged and neutralized by the antioxidants in the EOs studied. The radical scavenging activities were evaluated through a colorimetric procedure, where the purple-coloured DPPH• and green-coloured ABTS•+ would be converted to neutralized forms by protonation and electron transfer, respectively [20, 21].

As shown in Figure 5(a), KEO and BEO at 50 mg/mL concentrations achieved 7.26±1.28% and 77.55±1.57% DPPH radical scavenging activities, respectively. Nevertheless, only BEO demonstrated a significant dose-dependent increase in scavenging activity, allowing its IC_{50} value to be inferred. In contrast, KEO exhibited minimal scavenging activity across the concentration range tested and lacked an estimable IC_{50} value, as none of its tested concentrations reached the 50% scavenging threshold in this assay. A DPPH radical scavenging IC_{50} value of 41.7±0.1 mg/mL was previously reported for commercial kaffir lime EO [46]. In comparison, Höferl et al. [47] reported an IC_{50} value of 0.68 mg/mL for ginger rhizome EO from Ecuador, which shared citral as its main terpenoid with the BEO studied here. Warsito [48] estimated IC_{50} values ranging from 0.92 to 10.45 mg/mL for DPPH radical scavenging activity in commercially available ginger EOs from various sources. Comparing these findings, ginger EOs generally exhibited lower IC_{50} values than kaffir lime EOs, which correlated with their stronger scavenging abilities. This aligned with the results in Figure 5(a), where the BEO demonstrated higher DPPH radical scavenging activity across all tested concentrations, establishing it as a stronger DPPH scavenger than the KEO. The distinctly low performance of KEO observed here was potentially supported by Wungsintaweekul et al. [49], who reported an IC_{50} value exceeding 250 mg/mL for the DPPH scavenging activity of EO extracted from kaffir lime peels in Thailand, well above the concentration range (1–50 mg/mL) used in the present study.

Regarding the ABTS radical scavenging activity, both the EOs significantly reduced ABTS free radicals, showing strong dose-dependent behaviour and yielding inferable IC_{50} values for scavenging capacity. However, BEO emerged as the stronger ABTS scavenger, displaying higher scavenging percentages at most tested concentrations. The ABTS radical scavenging activities of KEO and BEO achieved 55.19±0.54% and 67.58±1.34% respectively at 50 mg/mL. These

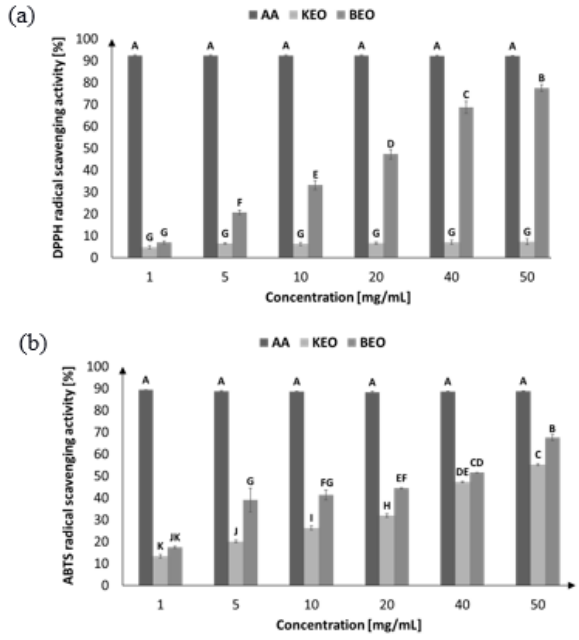

Fig. 5: Effects of KEO and BEO on the neutralization of DPPH (a) and ABTS (b) radicals. Ascorbic acid acts as the positive control.

Note: Bars with different alphabets are not significantly similar ($p < 0.05$)

findings aligned with the results of Ivanović et al. [18] and Saeio et al. [41], which reported that the ginger rhizome EO exhibited a TEAC (Trolox Equivalent Antioxidant Capacity) value close to 2, while the EO extracted from kaffir lime peels showed a lower TEAC value of 0.2 for the ABTS radical scavenging assay, respectively. The higher the TEAC value, the stronger the ABTS radical scavenging potential.

The overall higher DPPH and ABTS radical scavenging activities observed in the BEO could be attributed to its higher TPC and TFC values than the KEO. Hernández - Rodríguez et al. [36] have documented a direct correlation between phenolic content and DPPH and ABTS radical scavenging activities. This probably could be explained by the aromatic rings in phenolic and flavonoid compounds, which contain a conjugated system of double bonds that usually enhances radical stability during antioxidative scavenging reactions through resonance stabilisation within the rings [40]. Additionally, as discussed before, the greater abundance of oxygenated terpenoids in the BEO may further contribute to its superior radical scavenging capacity. Despite the KEO's underperformance in DPPH scavenging ability, it exhibited ABTS radical scavenging activity comparable to the BEO. This may suggest the presence of potent phenolic compounds in the KEO, even though its TPC and TFC were relatively lower. According to Wungsintaweekul et al. [49], flavonoids such as hesperidin contributed to the radical scavenging abilities in kaffir lime extracts. Furthermore, several primary and trace terpenes presented in the KEO, such as D-limonene, α-pinene, and linalool, had demonstrated ABTS radical scavenging potential, as noted by Wang et al. [39]. These compounds likely contributed to the KEO's effectiveness as a radical scavenger.

G. BSA denaturation inhibition capacity of EOs

Anti-BSA denaturation testing is a valuable in vitro method for assessing the anti-inflammatory potential of natural extracts, including EOs. This assay measures the ability of bioactive components to prevent heat-induced denaturation of BSA, a process in which proteins lose their secondary and tertiary structures, compromising their biological functions and potentially leading to the formation of autoantigens associated with inflammatory diseases like rheumatoid arthritis and systemic lupus erythematosus [22]. In the present study, the BSA denaturation inhibition assay was based on the ability of phytochemicals presented in the studied EOs to stabilise the BSA protein structure under induced heat stress. Stabilisation occurred with a reduction in precipitation contributing to turbidity, as the amount of structurally deformed BSA was decreased by the EOs presence. Turbidity intensity was then measured spectrophotometrically, where lower absorbance indicated a higher percentage of BSA denaturation inhibition.

As illustrated in Figure 6, KEO significantly inhibited the BSA denaturation by 69.59±0.16% and 70.69±0.22% at concentrations of 5 and 10 mg/mL, respectively, with no significant difference between these values. In contrast, BEO showed consistently low and statistically similar inhibitory activities across all the tested concentrations (0.5–10 mg/mL). By observing the overall trend in Figure 6, the KEO emerged as a stronger anti-inflammatory agent by demonstrating significantly higher BSA denaturation inhibition percentages than the BEO, especially at higher concentrations tested. The study observed the relatively low BSA denaturation inhibition activity for BEO despite its higher TPC and TFC values than the KEO. The absence of specific bioactive compounds in our BEO may explain this disparity. Funk et al. [50] reported that gingerols, key phenolic compounds commonly found in crude ginger rhizome extracts, could degrade during the heating process of hydrodistillation used for EO extraction, resulting in a gingerol-free EO. Gingerols and shogaols are primary phytocompounds responsible for the ginger extracts exerting potent anti-BSA denaturation activities [51]. The terpene profile of KEO, dominated by β-pinene, likely contributed to its enhanced inhibitory effect over BEO. Previous studies have demonstrated the anti-inflammatory properties of β-pinene through various mechanisms, including inhibiting pro-inflammatory mediators in a rat model [28, 29].

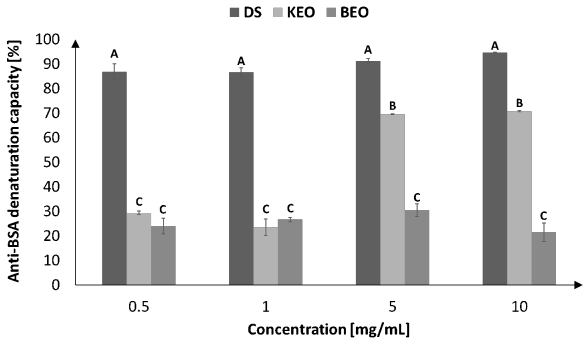

Fig. 6: Inhibitory effects of KEO and BEO towards BSA denaturation. Diclofenac sodium (DS) is the positive control used.

Note: Bars with common alphabets are not significantly different ($p < 0.05$)

V. CONCLUSION AND RECOMMENDATIONS

This study highlighted the distinct phytochemical profiles and bioactivities of EOs from kaffir lime peels and Bentong ginger rhizomes. KEO demonstrated superior anti-inflammatory and metal chelation properties, likely due to its β-pinene content, while BEO exhibited higher total phenolic and flavonoid contents, correlating with robust antioxidant activities. These findings suggested that KEO, derived from underutilized food waste, could serve as a more sustainable alternative over the BEO for industries focusing on natural products. The significant advances presented in this study included a standardized extraction method and the elucidation of key phytocompounds responsible for specific bioactivities. These insights provided a foundation for optimizing EO production and applications in pharmaceuticals, cosmeceuticals, and functional foods. Future research should focus on scaling up the extraction of KEO for commercial viability and exploring its synergistic effects with other EOs to enhance the bioactive performance. Investigating the mechanisms behind specific bioactive compounds, such as β-pinene, could also further broaden the aromatherapeutic potential of KEO. This work contributed to the growing demand for green consumerism on diverse natural oils, promoting economic and environmental benefits.

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REFERENCES

[1] Lubinska-Szczygeł M, Różańska A, Dymerski T, Namieśnik J, Katrich E, Gorinstein S. A novel analytical approach in the assessment of unprocessed Kaffir lime peel and pulp as potential raw materials for cosmetic applications. *In-*

dustrial Crops and Products. 2018;120: 313–321. https://doi.org/10.1016/j.indcrop.2018.04.036.

[2] Ghasemzadeh A, Jaafar HZE, Rahmat A. Identification and concentration of some flavonoid components in Malaysian young ginger (Zingiber officinale Roscoe) varieties by a high-performance liquid chromatography method. *Molecules*. 2011;15(9): 6231–6243. https://doi.org/10.3390/molecules15096231.

[3] Lizarraga-Valderrama LR. Effects of essential oils on central nervous system: Focus on mental health. *Phytotherapy Research*. 2020;35(2). https://doi.org/10.1002/ptr.6854.

[4] Barbieri C, Borsotto P. Essential oils: Market and legislation. In: Hany AES (ed.). *The potential of essential oils*. London: IntechOpen; 2018. p.107–127.

[5] Bingham LJ, Tam MM, Palmer AM, Cahill JL, Nixon RL. Contact allergy and allergic contact dermatitis caused by lavender: A retrospective study from an Australian clinic. *Contact Dermatitis*. 2019;81(1): 37–42. https://doi.org/10.1111/cod.13247.

[6] Lubinska-Szczygeł M, Kuczyńska-Łażewska A, Rutkowska M, Polkowska Z, Katrich E, Gorinstein S. Determination of the major by-products of Citrus hystrix peel and their characteristics in the context of utilization in the industry. *Molecules*. 2023;28(6): 2596. https://doi.org/10.3390/molecules28062596.

[7] Katerina V, Klara U, Samnang N, Ladislav K. Chemical composition of essential oils and supercritical carbon dioxide extracts from Amomum kravanh, Citrus hystrix and Piper nigrum "Kampot." *Molecules*. 2023;28(23): 7748. https://doi.org/10.3390/molecules28237748.

[8] Houng P, Ly K, Lay S. Valorization of kaffir lime peel through extraction of essential oil and process optimization for phenolic compounds. *Journal of Chemical Technology & Biotechnology*. 2023;98(11): 2745–2753. https://doi.org/10.1002/jctb.7354.

[9] González-Guevara JC, Madrigal-Redondo GL, Vargas-Zúñig R, Rodríguez-Sibaja S. Physicochemical characterization of medicinal essential oil from the rhizome of Zingiber officinale (ginger), grown in San Carlos, Costa Rica. *Journal of Applied Pharmaceutical Sciences–JAPHAC*. 2017;4(1): 9–18.

[10] Singh G, Kapoor IPS, Singh P, de Heluani CS, de Lampasona MP, Catalan CAN. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. *Food and Chemical Toxicology*. 2008;46(10): 3295–3302. https://doi.org/10.1016/j.fct.2008.07.017.

[11] Munda S, Dutta S, Haldar S, Lal M. Chemical analysis and therapeutic uses of ginger (Zingiber officinale Rosc.) essential oil: A review. *Journal of Essential Oil Bearing Plants*. 2018;21(4): 994–1002. https://doi.org/10.1080/0972060x.2018.1524794.

[12] Aqeel U, Aftab T, Khan MMA, Naeem M. Regulation of essential oil in aromatic plants under changing environment. *Journal of Applied Research on Medicinal and Aromatic Plants*. 2023;32: 100441. https://doi.org/10.1016/j.jarmap.2022.10044.

[13] da Silva Moura E, D'Antonino Faroni LR, Fernandes Heleno F, Aparecida Zinato Rodrigues A, Figueiredo Prates LH, Lopes Ribeiro de Queiroz ME. Optimal extraction of Ocimum basilicum essential oil by association of ultrasound and hydrodistillation and its potential as a biopesticide against a major stored grains pest. *Molecules*. 2020;25(12): 2781. https://doi.org/10.3390/molecules25122781.

[14] Liang J, Stöppelmann F, Schoenbach J, Rigling M, Nedele AK, Zhang Y, et al. Influence of peeling on volatile and non-volatile compounds responsible for aroma, sensory, and nutrition in ginger (*Zingiber officinale*). *Food Chemistry*. 2023;419: 136036. https://doi.org/10.1016/j.foodchem.2023.136036.

[15] National Institute of Standards and Technology. SRD 1A. *NIST Standard Reference Database 1A*. Gaithersburg: Mass Spectrometry Data Center; 2008.

[16] Sari KRP, Ikawati Z, Danarti R, Hertiani T. Microtiter plate assay for measurement of total phenolic and total flavonoid contents in medicinal plant extracts. *Arabian Journal of Chemistry*. 2023;16(9): 105003. https://doi.org/10.1016/j.arabjc.2023.105003.

[17] Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT*. 2021;150: 111932. https://doi.org/10.1016/j.lwt.2021.111932.

[18] Ivanović M, Makoter K, Islamčević Razboršek M. Comparative study of chemical composition and antioxidant activity of essential oils and crude extracts of four characteristic Zingiberaceae herbs. *Plants*. 2021;10(3): 501. https://doi.org/10.3390/plants10030501.

[19] Abdullah MZ, Mohd Ali J, Abolmaesoomi M, Abdul-Rahman PS, Hashim OH. Anti-proliferative, in vitro antioxidant, and cellular antioxidant activities of the leaf extracts from Polygonum minus Huds: Effects of solvent polarity. *International Journal of Food Properties*. 2017;20(1): 846–862. https://doi.org/10.1080/10942912.2017.1315591.

[20] Vilas-Boas AA, Campos DA, Nunes C, Ribeiro S, Nunes J, Oliveira A, et al. Polyphenol extraction by different techniques for valorisation of non-compliant Portuguese sweet cherries towards a novel antioxidant extract. *Sustainability*. 2020;12(14): 5556. https://doi.org/10.3390/su12145556.

[21] Benkhaira N, Koraichi SI, Fikri-Benbrahim K. In vitro methods to study antioxidant and some biological activities of essential oils: A review. *Biointerface Res. Appl. Chem*. 2022;12(3): 3332.

[22] Belkhodja H, Meddah B, Sidelarbi K, Bouhadi D, Medjadel B, Brakna A. In Vitro and in Vivo anti-inflammatory potential of Eucalyptus globulus es-

sential oil. *Journal of Applied Biological Sciences*. 2022;16(1): 80–88.

[23] Mohideen M, Idris MIH, Zainal Abidin NSI, Kamaruzaman NA. Review on extraction methods of essential oil from kaffir lime (*Citrus hystrix*) leaves. *Journal of Academia*. 2021;9: 173–184.

[24] Jahiddin FSA. *Chemical variability in volatile oils of Bentong ginger: A chemometric study*. Master's thesis. Malaysia: University of Malaya; 2020.

[25] Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (Zingiber officinale Roscoe). *International Journal of Current Pharmaceutical Research*. 2010;2(4): 40–43.

[26] Sharma S, Gupta J, Prabhakar PK, Gupta P, Solanki P, Rajput A. Phytochemical repurposing of natural molecule: Sabinene for identification of novel therapeutic benefits using in silico and in vitro approaches. *ASSAY and Drug Development Technologies*. 2019;17(8): 339–351. https://doi.org/10.1089/adt.2019.939.

[27] Van Zyl RL, Van Vuuren NCJ, Mustapha O. The isomeric effects of pinene on Plasmodium and Anopheles. *Proceedings for Annual Meeting of the Japanese Pharmacological Society*. 2018;WCP2018(0): PO1–9. https://doi.org/10.1254/jpssuppl.WCP2018.0_PO1-9-27.

[28] Santos ES, Abrantes Coelho GL, Saraiva Fontes Loula YK, et al. Hypoglycemic, hypolipidemic, and anti-inflammatory effects of beta-pinene in diabetic rats. *Evidence-Based Complementary and Alternative Medicine*. 2022;2022: 1–8.

[29] Yang JY, Choi WS, Kim KJ, Eom CD, Park MJ. Investigation of active anti-inflammatory constituents of essential oil from Pinus koraiensis (Sieb. et Zucc.) wood in LPS-stimulated RBL-2H3 cells. *Biomolecules*. 2021;11(6): 817. https://doi.org/10.3390/biom11060817.

[30] Sreepian A, Sreepian PM, Chanthong C, Mingkhwancheep T, Prathit P. Antibacterial activity of essential oil extracted from *Citrus hystrix* (Kaffir Lime) peels: An in vitro study. *Tropical Biomedicine*. 2019;36(2): 531–541.

[31] Gupta S, Pandotra P, Ram G, Anand R, Gupta AP, Husain MK, et al. Composition of a monoterpenoid-rich essential oil from the rhizome of Zingiber officinale from north western Himalayas. *Natural Product Communications*. 2011;6(1): 93–96. https://doi.org/10.1177/1934578x1100600122.

[32] Baschieri A, Ajvazi MD, Tonfack JLF, Valgimigli L, Amorati R. Explaining the antioxidant activity of some common non-phenolic components of essential oils. *Food Chemistry*. 2017;232: 656–663. https://doi.org/10.1016/j.foodchem.2017.04.036.

[33] Chen W, Viljoen AM. Geraniol – A review of a commercially important fragrance material. *South African Journal of Botany*. 2010;76(4): 643–651. https://doi.org/10.1016/j.sajb.2010.05.008.

[34] Venskutonis PR. Natural antioxidants in food systems. In: Grzegorz B (ed.). *Food oxidants and antioxidants: chemical, biological, and functional properties*. U.S.: CRC Press; 2013. p.235–288.

[35] Osae R, Apaliya MT, Kwaw E, Chisepo MTR, Yarley OPN, Antiri EA, Alolga RN. Drying techniques affect the quality and essential oil composition of Ghanaian ginger (*Zingiber officinale* Roscoe). *Industrial Crops and Products*. 2021;172: 114048. https://doi.org/10.1016/j.indcrop.2021.114048.

[36] Hernández-Rodríguez P, Baquero LP, Larrota HR. Flavonoids: Potential therapeutic agents by their antioxidant capacity. In: Campos MRS (ed.). *Bioactive Compounds*. Woodhead Publishing; 2019. p.265–288. https://doi.org/10.1016/b978-0-12-814774-0.00014-1

[37] Mohideen M, Mahadi NNSJ, Suhaimi NAN, Kamaruzaman NA, Azlan AYHN. Antibacterial properties of essential oil extracted from kaffir lime (*Citrus Hystrix*) peel. *Biomedical and Pharmacology Journal*. 2022;15(1): 179–186. https://doi.org/10.13005/bpj/2353.

[38] Deleanu M, Popa EE, Popa ME. Chemical composition and active properties evaluation of wild oregano (*Origanum Vulgare*) and ginger (*Zingiber Officinale-Roscoe*) essential oils. *Revista De Chimie*. 2018;69: 1927–1933.

[39] Wang HF, Yih KH, Huang KF. Comparative study of the antioxidant activity of forty-five commonly used essential oils and their potential active components. *Journal of Food and Drug Analysis*. 2020;18(1). https://doi.org/10.38212/2224-6614.2225.

[40] Adwas AA, Elsayed ASI, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. *Journal of Applied Biotechnology & Bioengineering*. 2019;6(1): 43–47. https://doi.org/10.15406/jabb.2019.06.00173.

[41] Saeio K, Chaiyana W, Okonogi S. Antityrosinase and antioxidant activities of essential oils of edible Thai plants. *Drug Discoveries & Therapeutics*. 2011;5(3): 144–149. https://doi.org/10.5582/ddt.2011.v5.3.144.

[42] Ling Q, Zhang B, Wang Y, Xiao Z, Hou J, Xiao C, et al. Chemical composition and antioxidant activity of the essential oils of citral-Rich chemotype Cinnamomum camphora and Cinnamomum bodinieri. *Molecules*. 2022;27(21): 7356. https://doi.org/10.3390/molecules27217356.

[43] Dhanik J, Verma A, Arya N, Nand V. Chemical profiling and antioxidant activity of essential oil of Zingiber officinale Roscoe from two different altitudes of Uttarakhand. *Journal of Essential Oil Bearing Plants*. 2017;20(6): 1547–1556. https://doi.org/10.1080/0972060x.2017.1417747.

[44] Irawaty W, Ayucitra A. Assessment on antioxidant

and in vitro antidiabetes activities of different fractions of *Citrus hystrix* peel. *International Food Research Journal*. 2018;25(6).

[45] Wojtunik-Kulesza KA, Wiśniewska R. Interactions of selected monoterpenes with iron and copper ions based on ferrozine and CUPRAC methods – The preliminary studies. *Chemistry & Biodiversity*. 2022;19(8). https://doi.org/10.1002/cbdv.202200461.

[46] Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. In vitro bioactivities of essential oils used for acne control. *International Journal of Aromatherapy*. 2006;16(1): 43–49. https://doi.org/10.1016/j.ijat.2006.01.006.

[47] Hoferl M, Stoilova I, Wanner J, Schmidt E, Jirovetz L, Trifonova D, et al. Composition and comprehensive antioxidant activity of ginger (*Zingiber officinale*) essential oil from Ecuador. *Natural Product Communications*. 2015;10(6): 1085–1090. https://doi.org/10.1177/1934578x1501000672.

[48] Warsito MF. Antibacterial and antioxidant activities of ginger essential oils. *Microbiology Indonesia*. 2021;15(4): 119–127. https://doi.org/10.5454/mi.15.4.119-127.

[49] Wungsintaweekul J, Sitthithaworn W, Putalun W, Pfeifhoffer HW, Brantner A. Antimicrobial, antioxidant activities and chemical composition of selected Thai spices. *Sonklanakarin Journal of Science and Technology*. 2010;32(6): 589.

[50] Funk JL, Frye JB, Oyarzo JN, Chen J, Zhang H, Timmermann BN. Anti-inflammatory effects of the essential oils of ginger (*Zingiber officinale* Roscoe) in experimental rheumatoid arthritis. *PharmaNutrition*. 2016;4(3): 123–131. https://doi.org/10.1016/j.phanu.2016.02.004.

[51] Ezzat SM, Ezzat MI, Okba MM, Menze ET, Abdel-Naim AB. The hidden mechanism beyond ginger (Zingiber officinale Rosc.) potent *in vivo* and *in vitro* anti-inflammatory activity. *Journal of Ethnopharmacology*. 2018;214: 113–123. https://doi.org/10.1016/j.jep.2017.12.019.