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Effectiveness of Zingiberaceae Herbal Extracts from the Hala-Bala Forest for Application in Thai Massage

Orawan Thipmanee[1], Sunee Waema[1], Saluma Samanman[2], Aeesoh Benhawan[1]*

[1] Major of Chemistry, Faculty of Science Technology and Agriculture, Yala Rajabhat University, Yala 95000, Thailand
[2] Faculty of Science and Technology, Princess of Naradhiwas University, Narathiwat 96000, Thailand

*Corresponding author email: aeesoh.b@yru.ac.th

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ABSTRACT. This research aimed to study the efficiency of essential oils extracted from Zingiberaceae herbals that are *Etlingera elatior*, *Zingiber montanum,* and *Etlingera coccinea,* collected from the Hala-Bala forest in Chulabhorn Pattana Village 9, Ban Santisuk 2, Mae Wat Sub-district, Than To and Betong District, Yala Province. The essential oils were extracted using steam distillation and characterized based on their physical and chemical properties, antimicrobial activity, phytochemical composition, and antioxidant activities. The pH values of the essential oils were found to be 6.1, 4.9, and 6.2, respectively. Heavy metal analysis of the essential oils, conducted using a flame atomic absorption spectrophotometer, revealed no contamination with lead, chromium, manganese, and cadmium. In antimicrobial activity test against three bacterial strains— *Staphylococcus aureus*, *Bacillus spp.,* and *Escherichia coli*—the essential oils of *E. elatior* and *Z. montanum* demonstrated inhibitory effects against all three strains, with inhibition zones of 12.3±0.28, 10.7±1.06, and 11.2±1.01 mm for *E. elatior* and 22.1±1.25, 26.0±0.70, and 18.5±0.70 mm for *Z. montanum*. Meanwhile, the essential oil of *E. coccinea* inhibited *S. aureus* and *E. coli* with inhibition zones of 20.5±0.70 and 13.2±0.35 mm, respectively. Preliminary phytochemical analysis was performed using high-performance thin-layer chromatography (HPTLC), and antioxidant activity evaluated using three assays: DPPH radical scavenging activity (DPPH assay), ABTS free radical bleaching (ABTS assay), and ferric reduction of antioxidant power (FRAP assay). The results confirmed that all three essential oils exhibited antioxidant activity, and the phytochemical screening detected flavonoids, diterpenes, and anthraquinones. This research highlights the potential of the essential oils for developing Thai massage health products, particularly in the form of massage oils infused with natural extracts and local herbs. Volunteer satisfaction assessments conducted at the Thai Traditional Medicine Learning Center, Yala Rajabhat University, Yala Province, Thailand, indicated a high to the highest level of satisfaction. This was attributed to the oils' natural composition, low toxicity, and high effectiveness.

Keywords: Antibacterial, Antioxidant, *Etlingera elatior*, Essential oil, *Etlingera coccinea*, Phytochemical, *Zingiber montanum*

INTRODUCTION

Plants with aromatic and medicinal properties have been used for various purposes, including adding flavor to food (Saffarionpour, 2024), medicinal applications (Parvin et al., 2023), preservation (Liu et al., 2024), beauty care (Anuradha and Bharadvaja, 2023), and stress relief products (Kate et al., 2023). These plants serve as a promising natural alternative, offering numerous benefits, safety, and sustainability. The therapeutic use of medicinal plants dates back to the earliest stages of human civilization. The herbal system of medicine is not only the oldest form of health care but also an essential component of modern civilization's development. Even today, a vast majority of the global population, particularly in the developing countries, continues to rely on herbal medicine and its products for primary health care needs.

Plants from the Zingiberaceae family, commonly known as ginger, are well-known for their strong aromatic and medicinal properties (Wahyuni et al., 2023). These plants are widely distributed throughout the tropics, particularly in Southeast Asia, and consists of 53 genera and over 1300 species (Pandey et al., 2023). The Zingiberaceae plants are commonly used in traditional medicine due to its various pharmacological and physiological effects. Studies have shown that their rhizomes are effective in treating several medical conditions, including digestive, respiratory, nervous, and muscular system problems, as well as degenerative diseases (Zulfadhly et al., 2023). The plant is used to treat a wide range of ailments such as nausea, vomiting, epilepsy, sore throat, coughs, colds, bruises, wounds, liver complaints, rheumatism, muscle aches, atherosclerosis, migraine headaches, high cholesterol, ulcers, and stomach discomfort (Pham et al., 2021). Phenolic compounds, secondary metabolites found in the rhizomes of Zingiberaceae plants, have been

shown to possess significant biological activity that benefits human health and have antibacterial properties useful in food and pharmaceutical applications (Alfuraydi et al., 2024; Mutakin et al., 2023; Rungruang et al., 2021). Zingiberaceae plants are rich in essential oils (EOs), which have important functions in nature and are commercially used in industries, including flavors, fragrances, cosmetics, agriculture, pharmaceuticals, health-care products, and others (Ivanovi´ et al., 2021). The primary objective is to replace synthetic products with natural EOs, which offer health benefits and also reduce adverse environmental impacts. Certain EOs have been found to possess potent antimicrobial, preservative, herbicidal, and antioxidant properties beneficial for many industries (Amil et al., 2024; Rawat et al., 2024; Vaishnavi et al., 2024).

In Thailand, the Hala-Bala forest complex is primarily tropical rainforest, with vegetation similar to that of northern Malaysia, which it borders. The species found in the southernmost provinces of Thailand's Hala-Bala forest, including *Etlingera elatior (E. elatior)*, *Zingiber montanum (Z. montanum),* and *Etlingera coccinea* (*E. coccinea*) are strongly aromatic herbs with medicinal properties. However, there are very few studies on the extraction of EOs from plants in the Hala-Bala forest area. Therefore, this presents an interesting and challenging opportunity for researchers in the region.

Most of the previously reported studies have shown that Zingiberaceae plants and their derivatives may be responsible for the antioxidant potential and antibacterial activity of ginger EO (Alfuraydi et al., 2024; Badrunanto et al., 2024; Gunasena et al., 2022; Mutlu-Ingok et al., 2021). However, the previous studies were limited to investigation of *E. elatior*, *Z. montanum,* and *E. coccinea* from the Hala-Bala forest, Thailand. Therefore, this research focuses on the effectiveness of EOs from Zingiberaceae plants, specifically *E. elatior*, *Z. montanum,* and *E. coccinea* from the Hala-Bala forest area in Chulabhorn Pattana Village 9, Ban Santisuk 2, Mae Wat Sub-district, Than To and Betong District, Yala Province. The physical and chemical properties of the EOs were determined, along with analysis of phytochemical compounds, antimicrobial activity and antioxidant properties. These EOs have also been developed into health products with traditional Thai massage in the form of massage oils containing EOs and oils from local herbs. This helps increase the value of local resources and provides another option for incorporating local herbs into traditional Thai medicine.

EXPERIMENTAL SECTION

Materials

Plant materials were collected from the Hala-Bala forest in Chulabhorn Pattana Village 9, Ban Santisuk 2, Mae Wat Sub-district, Than To and Betong Districts, Yala Province, Thailand. The rhizomes were separated from the plants, thoroughly cleaned, and prepared for use. Alpha-pinene, eucalyptol, and terpininen-4-ol were obtained from Sigma-Aldrich, USA. Nutrient agar and nutrient broth were purchased from Himedia, India.

Extraction of Essential Oils from *E. elatior*, *Z. montanum,* and *E. coccinea*

The extraction of EOs was studied using two methods: steam distillation — following a modified method described by Wainer et al., (2022) and boiling in coconut oil with steam, due to their simplicity and short extraction time. Moreover, these methods allow for the use of a large sample quantities, resulting in a higher yield of EO.

For the steam distillation method, the rhizomes of *E. elatior*, *Z. montanum,* and *E. coccinea* were peeled, thoroughly washed, and then cut into small pieces (1 × 1 cm). These pieces were subsequently dried for three hours. Afterward, 75 g of each rhizome sample was placed in a round-bottom flask and distilled with 500 mL of distilled water. The water was heated until it reached boiling point, producing steam, which rose and passed through the fresh rhizomes. As the steam carried the EOs through the system, it was directed into a condenser, where it cools and condensed back into liquid form. The EO layer was then carefully separated, transferred into an opaque vial, and stored at 4°C. The total volume of EOs extracted was measured and recorded. For the second method, freshly cut rhizome pieces were mixed with coconut oil at a 1:1 (% v/v) ratio and subjected to steam distillation for 30 minutes. Following the distillation process, the EOs were separated using a syringe, then stored in an opaque vial at 4 °C to maintain their stability and prevent degradation.

The pH values of the extracted EOs were determined using a pH meter (SI Analysis, Germany) which was calibrated with pH standard buffers before use to ensure accuracy. Additionally, the presence of heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr), and manganese (Mn), was analyzed using a flame atomic absorption spectrophotometer (Flame-AAS) (Shimadzu, Japan, AA 7000). The specific wavelengths used for detection were 283.3 nm for Pb, 228.8 nm for Cd, 367.9 nm for Cr, and 279.5 nm for Mn.

EO samples were digested using the wet digestion method. In a 100 mL beaker, 10 ml of nitric acid was added to 15 mL of EO sample. After digestion, the mixture was allowed to cooled, and 3 mL of 30% hydrogen peroxide was added. The beaker was covered, and the sample was gently heated to initiate the peroxide reaction. Subsequently, 5 mL of concentrated hydrochloric acid and 10 mL of deionized water were added, and the sample was further heated for 15 minutes without boiling. After cooling, the sample was filtered through a Whatman No. 5 filter paper and diluted to a final volume of 50 mL with deionized water.

Extraction of Essential Oils from Citronella Grass for Use as an Ingredient in Massage Oil Formulations

Citronella oil stimulates the muscles, nervous system, tissues, and skin, while helping to reduce aches and pains. It promotes blood circulation and can alleviate muscle spasms, back pain, sprains and cramps, as well as provide relief from fatigue or headaches. Therefore, it is used as an ingredient in herbal massage oil products. For EO extraction, washed samples and further chopped into small pieces and dried for 1 hour. Then 75 g of citronella and 150 mL of distilled water were distilled using the steam distillation method. After thorough distillation, the citronella EO was collected in an opaque vial and stored at 4 °C.

Antibacterial Testing

Antibacterial testing was performed following to the method described by Santoni et al., (2024). Three pathogenic bacteria strains were selected for this study to assess antimicrobial activities: *S. aureus*, *Bacillus* spp., and *E. coli*. These pathogens were obtained from the Medical and Industrial Microbiology program atYala Rajabhat University, Thailand. All bacterial cultures were maintained on nutrient agar (NA).

Bacteria colonies were transferred into 5 mL of nutrient broth (NB) and incubated at 37 °C for18-24 hours. The inocula were standardized with sterile NB to achieve a final cell concentration of 10^6-10^7 CFU/ml. The disc diffusion test was performed as described by Shimanuki and Knox (2000). An inoculum suspension of each bacterial strain was swabbed onto NA plates. Sterile 6 mm Whatman No. 1 filter paper discs were aseptically placed on plates. Then, 40 μL of volatile EOs were applied to the surface of the Whatman paper discs, which were cut into circles with a diameter of 6 mm for assay activity. After incubating the plates for 24-48 hours at 35-37 °C, the diameter of the inhibition zone was measured as an indicator of bacterial inhibition.

Phytochemical Preliminary Test

The qualitative phytochemical screening test of EO and herbal massage oil identified the main classes of active components —flavonoids, tannins, diterpenes, and anthraquinones — following a modified method described by Shaikh and Patil (2020).

The DPPH Assay for Radical Scavenging Activity was Performed Following to the Method Described by of Eugenio José Garcia et al. (2012).

A standard curve of Trolox in ethanol was prepared using various concentrations (10, 20, 30, 40, and 50 μM/mL), and the DPPH inhibition percentage for each concentration was calculated. A 0.5 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in 500 mL of ethanol. The EO extract was then mixed with the DPPH solution at a 9:1 volume ratio, thoroughly shaken, and kept in the dark at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The percentage of DPPH inhibition was calculated and expressed as micrograms of Trolox-equivalent antioxidant capacity (μg TEAC) per milliliter of sample.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Antioxidant Activity Analysis Using the ABTS Radical Cation Decolorization Assay

A standard curve of Trolox in ethanol was prepared using different concentrations (10, 20, 30, 40, and 50 μg/mL). The ABTS radical (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) was generated by mixing a 7 mM ABTS solution in ethanol (50 mL) with a 2.45 mM potassium persulfate solution in ethanol (25 mL) at a volume ratio of 2:1. The mixture was stored in the dark at room temperature for 16 hours. Subsequently, the EO extract was mixed with the ABTS solution at a volume ratio of 1:2 and left in the dark at room temperature for 5 min. The absorbance was then measured at a wavelength of 734 nm using a UV-Visible spectrophotometer. The percentage of $ABTS^{\bullet+}$ radical inhibition was calculated, and the results were expressed as micrograms of Trolox-equivalent antioxidant capacity per milliliter of sample.

$$\%ABTS^{\bullet+} \text{ Radical Inhibition} = [(A_{\text{control}} - A_{\text{test sample}})/A_{\text{control}}] \times 100$$

Antioxidant Activity Analysis Using Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was prepared by mixing an acetate buffer (pH 3.6) with a 20 mM ferric chloride solution ($FeCl_3 \cdot 6H_2O$) and a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid at a ratio of 10:1:1, respectively. Then, EO extract was then mixed with the FRAP reagent according to the details in Table 5. The mixture was shaken thoroughly and allowed to stand for 4 minutes before measuring the absorbance at a wavelength of 593 nm. The absorbance value was calculated using the equation:

$$\text{Absorbance} = A_{\text{test sample}} - A_{\text{blank}} - A_{\text{control}}.$$

The electron-donating capacity was determined by comparing the result to a standard curve of ferrous sulfate. The results were expressed as mM Fe^{2+} equivalents per gram of sample. (Benzie & Strain, 1996; Pulido et al., 2000).

HPTLC Analysis of Plant Extract

In the HPTLC analysis of plant extracts, a quantitative assessment of alpha-pinene, eucalyptol, and terpinen-4-ol was performed under specific chromatographic conditions. Standard solutions of alpha-pinene, eucalyptol, and terpinen-4-ol were prepared by dissolving 1 mg of each compound in 10 mL of methanol. Sample solutions, including EOs and herbal massage oil preparations, were prepared by dissolving 1 g of each sample in 10 mL of methanol. For the stationary phase, precoated silica gel 60 F254 plates (20 x 10 cm, Merck, Darmstadt, Germany) were used. The mobile phase for alpha-pinene and

eucalyptol consisted of a solvent mixture of *n*-hexane and ethyl acetate in a volume ratio of 8:2, while for terpinen-4-ol, a combination of toluene, ethyl acetate, and glacial acetic acid in a ratio of 8:2:0.3 was employed.

Sample application was carried out using a CAMAG Linomat-5, with 15 tracks per plate. Each band was 8 mm long, spaced 11.4 mm apart, positioned 8 mm from the lower edge, and the first application starting 20 mm from the left edge. Chromatogram development was conducted in an automatic developing chamber (CAMAG ADC-2) with chamber saturation for 20 minutes and conditioning at 33% relative humidity for 10 minutes using a saturated magnesium chloride solution. Plates were developed to a migration distance of 70 mm and dried for 5 minutes. Derivatization involved treatment with anisaldehyde-sulfuric acid reagent, followed by heating at 100°C for 5 minutes.

Quantitative evaluation of active compounds in EOs and herbal massage oils was performed using the CAMAG TLC Scanner 4. The concentrations of alpha-pinene, eucalyptol, and terpinen-4-ol in samples were determined by comparing the peak height or area of the chromatograms to reference standards. Calibration plots were generated by applying various volumes of standard solutions (1, 2, 3, 4, 5, 6, and 7 μL). Chromatographic analysis was conducted at a wavelength of 500 nm, and standard curves illustrating the relationship between the area under the curve and concentration were constructed. The linear relationship was expressed using the equation y = slope(x) + intercept, and the quantification of active compounds was performed using winCATS 1.2.6 software.

Preparation of Herbal Massage Oil Products

The incorporation of EOs and other herb components into herbal massage oil formulations has gained increasing popularity in recent years (Meha et al., 2024; Ningsih et al., 2023; Vora et al., 2024). This study investigated three herbal massage oil products formulated with EOs from *E. elatior*, *Z. montanum,* and *E. coccinea* together with other ingredients. Virgin coconut oil (100 mL) was measured using a digital scale and mixed menthol (100 ± 10 g), patchouli (75 ± 7.5 g), camphor (75 ± 7.5 g), wintergreen oil (50 ± 5 g), phlai oil (50 ± 5 g), turmeric oil (50 ± 5 g), and EOs (50 ± 5 g) from *E. elatior*, *Z. montanum,* and *E. coccinea*. The mixture was stirred until homogeneous using an overhead stirrer. The resulting massage oils were then stored in tightly sealed containers and subjected to a consumer preference testing.

RESULTS AND DISCUSSION

Extraction of the Essential Oils

In this work, two different methods—steam distillation and boiling in coconut oil with steam—were used to extract EOs from the rhizomes of *E. elatior*, *Z. montanum,* and *E. coccinea*. The results showed that the steam distillation method (**Figure 1**) yielded 5 mL of EO within 4 hours, which was 10 times higher than the yield obtained from the boiling method (0.5 mL). This is because plant materials are directly exposed to steam, which facilitates the release of EOs through evaporation. Additionally, steam distillation offers advantages such as simplicity and low cost, making it a more efficient extraction method.

Characterization of Essential Oils

pH Determination

pH can be defined as the amount of free hydronium ions in a solution and represents active acidity. The pH value of the EOs from *E. elatior, Z. montanum*, and *E. coccinea* were measured as 6.10 ± 0.05, 5.10 ± 0.11, and 6.20 ± 0.07, respectively. The differences in pH values of the EOs from the three Zingiberaceae herbs, especially the pH of *Z. montanum,* are due to variations in soil conditions suitable for their cultivation and growth. The optimal pH range for growing *E. elatior* and *E. coccinea* is between 6.0 and 7.0, while *Z. montanum* thrives in soil with a pH of 5.5–6.5. Therefore, soil conditions in different areas are key factors influencing the pH values of the EOs. However, all their pH values fall within the accepted standard range of the Industrial Product Standard that is set by ISO 1242:1999 (E). It was shown that the EOs were slightly acidic that can be used safely.

Figure 1. A typical steam distillation unit for extraction of the essential oils.

Heavy Metal Analysis

Herbal EOs from *E. elatior*, *Z. montanum,* and *E. coccinea* were analyzed using the wet digestion method. The results showed that none of the four heavy metals, namely Pb, Cd, Cr, and Mn were detected. During distillation in primitive stills or storage in opaque vials, metal impurities could not be released into EOs.

Antimicrobial Activity of Essential Oils

The ability of EOs from *E. elatior*, *Z. montanum,* and *E. coccinea* to inhibit bacteria was tested, as shown in **Table 1**. The inhibition of three bacterial strains—*S. aureus, Bacillus spp.,* and *E. coli*— was evaluated using disc diffusion method. *Z. montanum* exhibited the best inhibition against all three species of pathogenic bacteria, with inhibition zones of 22.1±1.25, 26.0±0.70, and 18.5±0.70 mm, respectively. *E. elatior* showed inhibition against all three species, with inhibition zones of 12.3±0.28, 10.7±1.06, and 11.2±1.01 mm. *E. coccinea* inhibited two species of pathogenic bacteria with inhibition zones of 20.5±0.70 and 13.2±0.35 mm, respectively.

Antimicrobial Activity of Herbal Massage Essential Oil Products

The antibacterial efficiency of herbal massage oil products derived from *E. elatior*, *Z. montanum* and *E. coccinea* was evaluated against three bacterial strains: *S. aureus*, *Bacillus* spp., and *E. coli* using the disc diffusion method. The results showed that the inhibition zones for *S. aureus* were 31.8±2.3, 33.5±1.0, and 33.2±0.3 mm, respectively. The inhibition zones for *Bacillus* spp. were 28.0±0.7, 30.1±0.5, and 29.5±2.2 mm, and for *E. coli*, the zones were 39.1±0.05, 40.3±0.1, and 39.6±0.2 mm. These results indicate that the herbal massage oils from *E. elatior*, *Z. montanum,* and *E. coccinea* are capable of inhibiting the growth of all three bacterial strains. Among these, the herbal massage oil derived from *Z. montanum* exhibited the highest antibacterial efficiency, followed by *E. elatior* and *E. coccinea*, respectively, as in **Table 2**. This is because its EO has a higher acidity level compared to the other two EOs, making it more effective in inhibiting bacterial growth. Additionally, *Z. montanum* contains a greater number of bioactive chemical compounds that contribute to its antibacterial properties compared to *E. elatior* and *E. coccinea.*

Preliminary Phytoconstituent Analysis

The preliminary phytoconstituent analysis of EOs and herbal massage oils from *Etlingera* and *Zingiber* species revealed both similarities and differences in bioactive compounds. As shown in **Table 3**, the EOs of *E. elatior, Z. montanum*, and *E. coccinea* contained flavonoids and diterpenes, which are known for their antioxidant and anti-inflammatory properties (Liga et al., 2023; Câmara et al., 2024; Vo et al., 2020). The herbal massage oils containing *E. elatior, Z. montanum*, and *E. coccinea* exhibited additional secondary metabolites, including tannins and anthraquinones. The EOs added to the herbal massage oils enhance the therapeutic potential, as flavonoids play a key role in relieving pain, chronic pain, and soreness associated with conditions like arthritis or muscle fatigue (Scuteri et al., 2021; Bako et al., 2023; Al-Khayri et al., 2022). In addition, diterpenes help reduce swelling, inflammation, and discomfort in muscles and joints. Their neuroprotective properties may contribute to managing nerve-related pain (Del Prado-Audelo et al., 2021; Habtemariam, 2023; Al-Khazaleh et al., 2024). Together, flavonoids and diterpenes offer a natural and holistic approach to managing pain, making them valuable components in traditional and modern therapeutic practices.

Table 1 The inhibition zone of Zingiberaceae herbal extracts (*E. elatior*, *Z. montanum*, *E. coccinea*) on pathogenic bacteria.

Pathogenic bacteria	Inhibition zone (mm.)			Control (70% Ethanol)
	E. elatior	*Z. montanum*	*E. coccinea*	
S. aureus	12.3±0.28	22.1±1.25	20.5±0.70	30.7±3.18
Bacillus spp.	10.7±1.06	26.0±0.70	ND	21.2±0.28
E. coli	11.2±1.01	18.5±0.70	13.2±0.35	22.8±1.04

*ND = Not detectable

Table 2 Inhibition zone of herbal massage essential oils products from *E. elatior*, *Z. montanum,* and *E. coccinea* on pathogenic bacteria

Pathogenic bacteria	Inhibition zone (mm.)			Control (70% Ethanol)
	E. elatior	*Z. montanum*	*E. coccinea*	
S. aureus	31.8±2.3	33.5±0.3	33.2±0.3	22.5±0.8
Bacillus spp.	28.0±0.7	30.1±0.5	29.5±2.2	18.8±2.7
E. coli	39.1±0.05	40.3±0.1	39.6±0.2	17.5±1.8

Table 3 Preliminary phytoconstituent analysis

Secondary Metabolites	Essential oils			Herbal massage oil products			Test
	E. elatior	*Z. montanum*	*E. coccinea*	*E. elatior*	*Z. montanum*	*E. coccinea*	
flavonoid	+	+	+	+	+	+	Alkaline reagent test
tannins	-	-	-	+	+	+	Braymer's test
diterpenes	+	+	+	+	+	+	Copper acetate test
Anthraquinones	-	-	-	+	+	+	Borntrager's test

Table 4 Results of linear regression analysis for the calibration of alpha-pinene, eucalyptol, and terpinen-4-ol

Standard substance	Mobile phase	R_f	Linearity range (ng/band)	Regression equation	Correlation coefficient
Alpha-pinene	n-hexane : ethyl acetate (8:2)	0.19	100 - 700	y = 0.8464x + 456.64	0.9977
Eucalyptol		0.52	100 - 700	y = 11.075x + 1756	0.9902
Terpinen-4-ol	toluene : ethyl acetate : glacial acetic acid (8:2:0.3)	0.25	100 - 700	y = 16.689x + 487.69	0.9922

Antioxidant Activity Assays and HPTLC Analysis of Active Compounds.

The linearity of the three standards was observed within the range of 100–700 ng/band (**Table 4**). The peak areas for seven concentrations of alpha-pinene, eucalyptol, and terpinen-4-ol were calculated, and individual calibration curves were plotted for each compound by concentration versus peak area. The regression equations for alpha-pinene, eucalyptol, and terpinen-4-ol were determined to be y = 0.8464x + 456.64, y = 11.075x + 1756, and y = 16.689x + 487.69. The correlation coefficients (r) were 0.9977, 0.9902, and 0.9922, indicating strong linearity for each compound.

The DPPH, ABTS, and FRAP assays provided insights into the antioxidant capacities of EOs from *Etlingera* and *Zingiber* species, as well as their respective herbal massage oil formulations. From Table 5, for the DPPH assay, which measures free radical scavenging, *E. coccinea* displayed the highest antioxidant capacity (556.44 μg Trolox/mL), slightly higher than *Z. montanum* (551.47 μg Trolox/mL) and *E. elatior* (547.77 μg Trolox/mL). When EOs are added to a massage oil formula, the antioxidant activity was significantly enhanced, indicating the contribution of EOs to the overall antioxidant potential. The ABTS assay, which also evaluates free radical inhibition, showed *Z. montanum* to having the highest inhibition (585.31 μg Trolox/mL), followed by *E. elatior* (382.41 μg Trolox/mL) and *E. coccinea* (375.29 μg Trolox/mL). These results align with the DPPH assays, underscoring the strong antioxidant properties of *Z. montanum*. When these EOs were incorporated into massage oils, *Z. montanum* maintained the highest inhibition (525.11 μg Trolox/mL), followed by *E. elatior* (458.51 μg Trolox/mL) and *E. coccinea* (436.39 μg Trolox/mL). This suggests that *Z. montanum's* robustness as an antioxidant remains even in diluted or mixed formulations. In the FRAP assay, which measures the reducing power (ability to donate electrons), *Z. montanum* displayed a notably high antioxidant value (79,505.88 μg Fe^{2+}/g), far exceeding *E. elatior* (1,905.88 μg Fe^{2+}/g) and *E. coccinea* (1,305.88 μg Fe^{2+}/g). When added to herbal massage oils, the FRAP values of all formulations increased, with *Z. montanum* again leading (179,141.2 μg Fe^{2+}/g), followed by *E. elatior* (157,611.8 μg Fe^{2+}/g) and *E. coccinea* (90,670.59 μg Fe^{2+}/g). However, the base oil without EOs exhibited a substantial FRAP value (67,131.89 μg Fe^{2+}/g) because the massage oil formula contains various herbal ingredients. All assays demonstrated that *Z. montanum* exhibited the strongest antioxidant potential in both pure EOs and those incorporated into massage oil formulations, particularly in the FRAP assay, with its exceptionally high reducing power. Both the DPPH and ABTS assays confirmed that *Z. montanum* and *E. coccinea* have potent free radical inhibition capabilities, with *Z. montanum* maintaining its antioxidant capacity even when added to a massage oil formulation.

The HPTLC analysis of EOs and herbal massage oils from *Etlingera* and *Zingiber* species revealed the presence of alpha-pinene, eucalyptol, and

terpinen-4-ol. In the EOs, *E. elatior* contained alpha-pinene at 589.76 ng (0.122%) and eucalyptol at 425.65 ng (0.089%). *Z. montanum* had alpha-pinene at 434.56 ng (0.091%), eucalyptol at 432.56 ng (0.090%), and terpinen-4-ol at 246.39 ng (0.061%). *E. coccinea* showed alpha-pinene at 424.94 ng (0.082%), eucalyptol at 241.56 ng (0.059%), and terpinen-4-ol at 239.67 ng (0.055%). In herbal massage oils with EOs added, the levels of these compounds generally increased. For *E. elatior*, alpha-pinene was measured at 611.56 ng (0.086%), eucalyptol at 441.56 ng (0.065%), and terpinen-4-ol at 145.56 ng (0.019%). *Z. montanum* exhibited increased yields with alpha-pinene at 678.56 ng (0.092%), eucalyptol at 616.64 ng (0.088%), and terpinen-4-ol at 345.67 ng (0.051%). *E. coccinea* contained alpha-pinene at 638.56 ng (0.090%), eucalyptol at 412.57 ng (0.067%), and terpinen-4-ol at 422.65 ng (0.069%). In the herbal massage oil without added EOs, lower levels of these compounds were detected: alpha-pinene at 231.46 ng (0.039%), eucalyptol at 249.78 ng (0.042%), and terpinen-4-ol at 145.85 ng (0.020%). The HPTLC analysis provides an understanding of the presence and concentrations of specific bioactive compounds (alpha-pinene, eucalyptol, and terpinen-4-ol) in EOs and herbal massage oils derived from *Etlingera* and *Zingiber* species. Each component has documented therapeutic properties: alpha-pinene is known for its anti-inflammatory and bronchodilator effects (Salehi et al., 2019), eucalyptol for its potential as an anti-inflammatory and antimicrobial agent (Hoch et al., 2023), and terpinen-4-ol for its antioxidant and antimicrobial benefits (Badr, et al., 2023). The antioxidant mechanism of alpha-pinene and eucalyptol involves stabilizing and preventing the further oxidation of unstable free radicals, such as hydroxyl radicals (•OH), superoxide anions (O_2•$^-$), and peroxyl radicals (ROO•), by donating hydrogen atoms (H•). (Xu et al., 2019). In EOs, *Z. montanum* displayed the highest concentrations across all compounds, especially in alpha-pinene (434.56 ng) and eucalyptol (432.56 ng), suggesting strong antioxidant and antimicrobial potentials. *E. elatior* and *E. coccinea* also showed significant yields, although lower than *Z. montanum*. When incorporated into massage oils, the levels of these components increased substantially across all three species, likely due to the presence of EOs. Specifically, *Z. montanum* in the massage oil formulation retained the highest concentrations, with alpha-pinene reaching 678.56 ng and eucalyptol at 616.64 ng.

Characteristics and Volunteer Satisfaction Test Used of the Herbal Massage Oil

The observation of the color parameter showed a yellow result, as displayed in **Figure 2**. This is due to the use of Phlai and turmeric oil, which contains curcuminoids that can give rise to a yellow-lemon color in the formula. All the herbal massage oils were clear and in the form of homogeneous liquids, due to the good solubility of the EOs and the carrier oil, virgin coconut oil. The good solubility is attributed to the similar polarity of the oils. In testing the efficiency and satisfaction of volunteers at the Thai massage clinic, Yala Rajabhat University, Thailand, who tested three of herbal massage oils, it was found that all 33 volunteers had satisfaction levels from herbal massage oil from *E. elatior*, *Z. montanum and E. coccinea* EO were 4.52 ± 0.63, 4.60 ± 0.57 and 4.40 ± 0.65, respectively. The highest level of overall satisfaction

Table 5 Results of antioxidant activity assays and HPTLC analysis of active compounds.

Sample	Antioxidant activity assay			HPTLC method		
	DPPH (μg Trolox/mL)	ABTS (μg Trolox/ mL)	FRAP value (μg Fe^{2+}/g)	alpha - pinene (ng, % yield)	Eucalyptol (ng, % yield)	Terpinen-4-ol (ng, % yield)
Essential oils						
E. elatior	547.77	382.41	1,905.88	589.76 (0.122)	425.65 (0.089)	-
Z. montanum	551.47	585.31	79,505.88	434.56 (0.091)	432.56 (0.090)	246.39 (0.061)
E. coccinea	556.44	375.29	1,305.88	424.94 (0.082)	241.56 (0.059)	239.67 (0.055)
Herbal massage oil products						
E. elatior	382.76	458.51	157,611.8	611.56 (0.086)	441.56 (0.065)	145.56 (0.019)
Z. montanum	559.60	525.11	179,141.2	678.56 (0.092)	616.64 (0.088)	345.67 (0.051)
E. coccinea	426.53	436.39	90,670.59	638.56 (0.090)	412.57 (0.067)	422.65 (0.069)
No add	328.24	323.10	67,131.89	231.46 (0.039)	249.78 (0.042)	145.85 (0.020)

Figure 2. Herbal massage oil containing the EOs of *E. elatior* (**A**), *Z. montanum* (**B**) and *E. coccinea* (**C**)

with the massage oil made from *Z. montanum* EOs, followed by *E. elatior* and *E. coccinea* EOs, respectively. This is because when aroma massage is applied to the skin and absorbed into the bloodstream through the skin pores, it provides a feeling of comfort and reduces the need for invasive methods of pain relief. Moreover, massage therapy using EOs is easier, cheaper, and has no side effects.

CONCLUSIONS

The Zingiberaceae plants also contain valuable EOs that are useful in various fields, primarily due to their strong odor and the wide range of pharmacological effects they possess. The main constituents of EOs are diterpenes, which exhibit pronounced antibacterial and antioxidant effects. In this study, we successfully isolated the EOs of three plant species: *E. elatior*, *Z. montanum*, and *E. coccinea* using the green distillation method. The results confirmed that the antimicrobial effect was observed with inhibition zones of 22.1±1.25, 26.0±0.70, and 18.5±0.70 mm against three strains: *S. aureus*, *Bacillus spp.*, and *E. coli*. Regarding antioxidant capacity, alpha-pinene, eucalyptol and terpinen-4-ol were the main diterpenes present in all the EOs studied, and these compounds also exhibit therapeutic properties. Additionally, the application of massage oils containing EOs was found to be highly effective and was considered safe for customers, as they were free from toxic substances. Herbal massage oil from *E. montanum* is the most suitable use for the massage oil. Recommendations for future work include analyzing the chemical composition of essential oils (EOs) using the GC-MS technique.

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