

EXPLORING THE THERAPEUTIC POTENTIAL: METABOLITE PROFILING, ANTIBACTERIAL ACTION, AND ANTIAGING PROPERTIES OF ESSENTIAL OILS FROM *CURCUMA MANGGA*, *BOESENBERGIA ROTUNDA*, AND *ZINGIBER PURPUREUM* RHIZOMES

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ABSTRACT

Objective: Bacterial infections and aging are common skin issues, with antibiotics and chemical treatments often causing side effects. Essential oils from Zingiberaceae rhizomes could offer safer antibacterial and anti-aging alternatives. This study aims to determine the metabolite profile of essential oils from three Zingiberaceae species (*C. mangga*, *B. rotunda*, and *Z. purpureum*) and assess their antibacterial and anti-aging properties.

Methods: Essential oils were extracted from fresh rhizomes via hydrodistillation. Metabolite profiling was conducted using FTIR and GC-MS techniques. Antibacterial activity was assessed through the microdilution method, and anti-aging activity was measured using tyrosinase and collagenase enzyme inhibition assays.

Results: The main components of *C. mangga* essential oil were β -Myrcene, L- β -Pinene, and Cineole; *B. rotunda* oil contained Champor, Ocimene, and Geraniol; and *Z. purpureum* oil had terpinene-4-ol, β -phellandrene, and terpinolene. Minimum Inhibitory Concentration (MIC) values for *C. mangga*, *B. rotunda*, and *Z. purpureum* essential oils were 100, 100, and 25 mg/ml against Gram-positive *S. aureus*, and 200, 100, and 12.5 mg/ml against Gram-negative *E. coli*. Minimum bactericidal concentration (MBC) values were similar to MIC values. The IC₅₀ values for anti-tyrosinase were 1.283, 2.897, and 12.028 mg/ml for *C. mangga*, *B. rotunda*, and *Z. purpureum*, respectively. None of the essential oils inhibited collagenase activity.

Conclusion: Essential oils from *C. mangga*, *B. rotunda*, and *Z. purpureum* rhizomes exhibited potential as anti-aging agents through tyrosinase inhibition. Still, they demonstrated weak antibacterial activity and did not inhibit collagenase enzymes.

Keywords: Essential oil, FTIR, GC-MS, Antibacterial, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC), Anti-aging

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INTRODUCTION

The skin is the body's outermost protective layer, defending against harmful microorganisms, mechanical stress, extreme temperatures, toxins, and radiation [1]. Like other tissues, the skin also undergoes aging processes, which can be classified as intrinsic or extrinsic [2]. Intrinsic aging occurs with age and is characterized by fine wrinkles and thinning of the epidermis. In contrast, extrinsic aging is caused by chronic sun exposure and is characterized by deep wrinkles, sagging skin, and hyperpigmentation [3].

As the skin ages, its structure and functioning change, affecting its protective abilities and wound healing and causing pigment changes and skin cancer [4]. Although the aging process is natural, there are methods to slow it down. Common anti-aging agents like hydroquinone and kojic acid are effective but known to be carcinogenic. Therefore, there are continuous efforts to develop anti-aging compounds that are safe and less toxic [5, 6].

On the other side, the problem of bacterial resistance to antibiotics is still a global health problem [7]. Several antibiotic-resistant bacteria have been found including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE), Penicillin-Resistant Pneumococci, *Klebsiella pneumoniae* that produces Extended-Spectrum Beta-Lactamase (ESBL), Carbapenem-Resistant *Acinetobacter baumannii* and Multiresistant *Mycobacterium tuberculosis* [8]. Multidrug-resistant *Staphylococcus aureus* is one of the major organisms causing bloodstream infections which are associated with high morbidity and mortality, which warrants the development and introduction of newer antimicrobial agents [9].

In light of these issues, there is a growing demand for natural alternatives in antibacterial and anti-aging compounds [10, 11]. Essential oils, which have antibacterial, immunomodulatory, and anti-aging properties, are sourced from nature. These oils are

volatile aromatic mixtures containing terpenoids synthesized by the mevalonate pathway, phenolic compounds derived from the shikimate pathway, saturated and unsaturated hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, and phenol oxides [12, 13]. Terpenoid class compounds have antibacterial and anti-aging properties, contributing to skin protection [14, 15].

Several studies have been conducted to assess the antibacterial activity of essential oils extracted from the rhizomes of *Curcuma mangga* Valeton and Zijp, *Boesenbergia rotunda* L. Mansf, and *Zingiber purpureum* Roscoe [16–18]. However, the activity can vary depending on factors such as the place of growth or origin, variety or cultivar, growing conditions, freshness of rhizomes, extraction method, solvent used, and the active components present [19]. Some essential oils from the Zingiberaceae family also have antioxidant and anti-inflammatory properties that can help fight premature aging [20–22]. However, further investigation is needed to evaluate the antibacterial and anti-aging effects of three rhizome essential oils: *Curcuma mangga* Valeton and Zijp, *Boesenbergia rotunda* L. Mansf, and *Zingiber purpureum* Roscoe.

MATERIALS AND METHODS

Plant collection

The rhizomes of *Curcuma mangga* Valeton and Zijp from Pariaman city, meanwhile *Boesenbergia rotunda* L. Mansf and *Zingiber purpureum* Roscoe were collected from Padang city, West Sumatra, Indonesia. Subsequently, plant identification was conducted at the Herbarium Andalas, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

Essential oils extraction

Fresh rhizomes were washed and dried at room temperature. The rhizomes were then chopped to a thickness of 2-3 mm. A sample of 9.2

kg, 8.9 kg, and 9 kg of *C. mangga*, *B. rotunda*, and *Z. purpureum* rhizomes were distilled using the hydrodistillation method for 6 h. The essential oil obtained was added to anhydrous sodium sulfate to remove the remaining water. Then, the essential oil was stored at 4 °C until used.

FTIR spectrum measurement

The FTIR spectra were obtained using a Thermo Scientific Nicolet iS10 spectrometer. The sample was placed on the ATR surface and scanned at a wave number range of 4000-650 cm⁻¹ at a temperature of 25 °C. The scan was carried out with 32 scans and a resolution of 8 cm⁻¹. The resulting spectrum was adjusted to the previously measured background air. Each spectrum measurement was repeated three times. The collected IR spectrum was preprocessed

using OMNIC software, including Atmospheric Correction and Smoothing. Finally, the functional groups were identified based on the literature [23].

Analysis of essential oils using gas chromatography-mass spectrometry (GC-MS)

The chemical components of essential oils extracted from the rhizomes of *C. mangga*, *B. rotunda* and *Z. purpureum* were identified using Gas Chromatography-Mass Spectrometry (GC-MS) with the following conditions (table 1). The peaks obtained were identified using the "NIST library". Fragmentation patterns were compared with previously published articles and the NIST Chemistry Webbook to confirm peak identification [23].

Table 1: The GC-MS condition

Parameter	Condition
GC Condition Inlet	
Heater	250 °C
Pressure	11.7 psi
Split ratio	200: 1
Split flow	240 ml/min
Columns	HP-5MS (Agilent®)
Flow rate	1.2 ml/min
Pressure	11.7 psi
Oven	Initial temperature: 80 °C Initial time: 1 min
Temperature program	80-100 °C at a rate 2 °C/min. 110-140 °C at a rate 3 °C/min. 140-170 °C at a rate 4 °C/min. 170-200 °C at a rate 2 °C/min
MS Condition	MS Source: 230 °C MS Quad: 150 °C Tune type: EI

Antibacterial activity test

Test microorganism

The test bacteria used in this study were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, obtained from the Padang Health District Laboratory.

Antibacterial activity

The Minimum Inhibitory Concentration (MIC) was determined using the microdilution method. First, the essential oil was diluted using DMSO to create a stock solution with a concentration of 20%. Then, serial dilution was performed to achieve 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml concentrations. Afterward, 50 µl of bacterial inoculum with a 10⁶ CFU/ml concentration was added to all wells and incubated for 18 h at 37 °C. About 40 µl of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) at a concentration of 0.5 mg/ml was added to each well and incubated for 15 min at 37 °C. The color change from colorless to purple indicated the MIC value. All samples, negative and positive controls, were tested in triplicates [22, 23]. The medium from each well was collected using a sterile cotton bud, streaked onto nutrient agar, and then incubated for 24 h at 37 °C. The minimum bactericidal concentration (MBC) was determined by observing the absence of bacterial growth on the streak or by identifying the lowest concentration that can kill 99.9% of the bacteria [24, 25].

Anti-tyrosinase activity

Test solutions of essential oil were prepared at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.156 mg/ml. Kojic acid at concentrations of 1.25, 0.625, 0.3125, 0.156, 0.079, 0.039 µg/ml was used as the positive control. First, 80 µl** of 50 mmol phosphate buffer solution at pH 6.5 was dispensed into the wells, followed by the addition of 40 µl** of the test solution. Subsequently, 40 µl** of tyrosinase enzyme (250 units/ml) was added, and the mixture was incubated at room temperature for 5 min. Following this, 40 µl** of 5.07 mmol L-DOPA substrate was added to each well. The 96-well microplate was then placed in an incubator at 30°C for 30 min. The dopachrome formed was measured using a microplate reader at a wavelength of 492 nm. The percentage of tyrosinase inhibition was

calculated as follows: [26]

$$\% \text{ Inhibition} = \frac{(A-B)}{A} \times 100\%$$

Notes:

A: Absorbance of negative control

B: Absorbance of sample

Anti-collagenase activity

The anti-collagenase activity was conducted using Sigma Aldrich's Collagenase Activity Colorimetric Assay Kit. In each well of a 96-well microtiter plate, 5 µl** of essential oil solution (5, 2.5, 1.25, and 0.625 mg/ml) was added, followed by 10 µl** of the provided collagenase kit (0.35U/ml), and then diluted to 100 µl** with Collagenase Assay Buffer. Then, 10 µl** of the provided collagenase kit (0.35U/ml) was added for the negative control. For the Inhibitor Control, 10 µl** of the provided collagenase kit (0.35U/ml) and 5 µl** of inhibitor (1,10-Phenanthroline) were added to the wells and diluted to 100 µl** with Collagenase Assay Buffer. The blank contained 100 µl** of Collagenase Assay Buffer only. Then, 100 µl** of a mixture of 40 µl** collagenase substrate (FALGPA) and 60 µl** Collagenase Assay Buffer were added into each well and incubated for 15 min at 37 °C. Absorbance was measured at 345 nm for 15 min. The percentage of inhibition was calculated using the following equation: [27]

$$\% \text{ Inhibition} = \frac{(A-B)}{A} \times 100\%$$

Notes:

A: Absorbance of sample

B: Absorbance of negative control

RESULTS

Physical characteristics

The physical characteristics of *C. mangga*, *B. rotunda* and *Z. purpureum* essential oil included yield, color, refractive index, and specific gravity, were listed in table 2. Fig. 1 shows the rhizome of *C. mangga*, *B. rotunda* and *Z. purpureum*.



Fig. 1: The rhizomes of (a) *Curcuma mangga* valetton and zipp (b) *Boesenbergia rotunda* L. mansf (c) *Zingiber purpureum* roscoe

Table 2: Physical characteristics of *C. mangga*, *B. rotunda* and *Z. purpureum* essential oils

Physical characteristic	Yield (%w/v)	Color	Specific gravity (g/ml)	Refractive index
<i>Curcuma mangga</i> Valetton and Zipp	0.143	Clear yellow	0.819	1.4733±0.0003
<i>Boesenbergia rotunda</i> L. Mansf	0.218	Clear yellow	0.89	1.4796±0.0006
<i>Zingiber purpureum</i> Roscoe	0.24	Clear yellow	0.91	1.4884±0.0003

Refractive index data showed as mean±SD (n=3); where n is the number of observations.

Essential oils' quality and value are determined by their specific gravity, refractive index, and water content. Essential oils of *C. mangga*, *B. rotunda*, and *Z. purpureum* have a distinct odor and clear yellow color, varying yields of 0.819% b/v, 0.89% b/v, and 0.91% b/v, respectively. The specific gravity describes the weight of the compound components, while the constituent components can influence the refractive index value. The water content in essential oils influences their refractive index, as water can refract light. Factors affecting essential oil quality include differences in plant origin, plant age at harvest, time of plant collection, material

treatment before, during, and after distillation, method of extraction, and human error [28].

FTIR spectroscopy analysis

Fig. 2 displays the FTIR spectra of *C. mangga*, *B. rotunda*, and *Z. purpureum* essential oil in the Middle Infra-Red (MIR) region. The peaks in the FTIR spectra corresponded to specific functional groups that absorbed infrared radiation. Each functional group absorbed IR radiation at a specific wavenumber, resulting in the appearance of peaks at those particular wavenumbers (table 3).

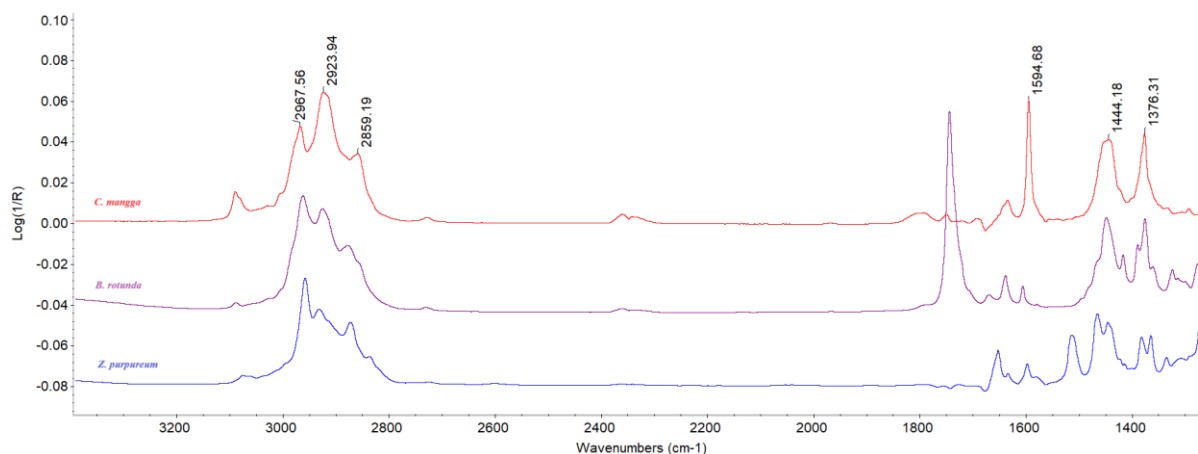


Fig. 2: FTIR Spectra of *C. mangga*, *B. rotunda* and *Z. purpureum* essential oils

Table 3: The functional group that absorbs infrared light at wavenumbers 4000–650 cm⁻¹

Wavenumber (cm ⁻¹)	Functional group
2967-2859	Asymmetrical and symmetrical stretching vibration of methylene (-CH ₂) group
1743	C=O stretching vibration
1652-1634	C=C stretching vibration
1444-1376	Bending vibrations of methyl (-CH ₃) dan methylene (CH ₂)

The spectra revealed significant peaks at 2967-2859 cm⁻¹ attributed to symmetric and antisymmetric C-H stretching of alkane chain CH₂ and CH₃ groups in the rhizome essential oils of *C. mangga*, *B. rotunda*, and *Z. purpureum*. The peak at 1743 cm⁻¹ indicated the presence of the

stretching vibration of the C=O group, found in the essential oil of *B. rotunda*. Additionally, a peak at wave number 1652-1634 cm⁻¹ indicated the presence of C=C alkenes in both essential oil samples. C-H bending was observed at wave numbers 1444-1376 cm⁻¹.

Chemical compositions of *C. mangga*, *B. rotunda* and *Z. purpureum* essential oil

The chemical compositions of the essential oil derived from *C. mangga*, *B. rotunda*, and *Z. purpureum* rhizomes are shown in table 4. The essential oil of *C. mangga* was mainly composed of terpenoid

compounds, accounting for 89.968% of the total detected by GC-MS. The predominant components in the essential oil of *B. rotunda* were oxygenated monoterpene (69%) and hydrocarbon monoterpene (31%). In the essential oil of *Z. purpureum*, 23 components were identified, with terpenoid compounds constituting 87.34%, including monoterpenes at 85.69% and sesquiterpenes at 1.65%.

Table 4: Chemical composition of *C. mangga*, *B. rotunda* and *Z. purpureum* essential oils

Chemical compound	Molecular weight	Molecular formula	Percentage of Relative Area (%)		
			<i>C. mangga</i>	<i>B. rotunda</i>	<i>Z. purpureum</i>
β-Myrcene	136.125	C ₁₀ H ₁₆	29.402	1.088	-
L-β-Pinene	136.125	C ₁₀ H ₁₆	20.916	-	-
Cineole	154.136	C ₁₀ H ₁₈ O	10.056	-	-
α-Pinene	136.125	C ₁₀ H ₁₆	5.599	0.742	2.72
β-cis-Ocimene	136.125	C ₁₀ H ₁₆	5.390	4.036	-
Germacrone	218.167	C ₁₅ H ₂₂ O	3.056	-	-
(+)-Camphor	152.120	C ₁₀ H ₁₆ O	2.131	-	-
D-Limonene	136.125	C ₁₀ H ₁₆	1.274	1.515	-
Caryophyllene	204.188	C ₁₅ H ₂₄	1.265	-	-
Terpineol	154.136	C ₁₀ H ₁₈ O	1.260	1.296	-
Caryophyllene oxide	220.183	C ₁₅ H ₂₄ O	1.073	-	-
m-Camphorene	272.250	C ₂₀ H ₃₂	0.936	-	-
Camphene	136.125	C ₁₀ H ₁₆	0.913	-	-
Terpine-4-ol	154.136	C ₁₀ H ₁₈ O	0.889	0.336	30.30
β-Selinenol	222.198	C ₁₅ H ₂₆ O	0.882	-	-
Camphor	154.136	C ₁₀ H ₁₈ O	0.652	28.433	-
Isoborneol	154.136	C ₁₀ H ₁₈ O	0.650	-	-
Selinenol	222.198	C ₁₅ H ₂₆ O	0.585	-	-
Curcumenone	234.162	C ₁₅ H ₂₂ O ₂	0.474	-	-
p-Camphorene	272.250	C ₂₀ H ₃₂	0.440	-	-
Curcumenol	234.162	C ₁₅ H ₂₂ O ₂	0.437	-	-
Ambrial	234.198	C ₁₆ H ₂₆ O	0.418	-	-
Epicurzerenone	230.131	C ₁₅ H ₁₈ O ₂	0.367	-	-
2-Isopropyl-5-methyl-9-methylene-bicyclo-1-decene(4.4.0)	204.188	C ₁₅ H ₂₄	0.348	-	-
2-Nonanone	142.136	C ₉ H ₁₈ O	0.345	-	-
β-Sabinene	136.125	C ₁₀ H ₁₆	0.337	-	-
Selinenol	222.198	C ₁₅ H ₂₆ O	0.337	-	-
β-Elemene	204.188	C ₁₅ H ₂₄	0.299	-	-
Ocimene	136.125	C ₁₀ H ₁₆	-	16.689	1.12
Geraniol	154.136	C ₁₀ H ₁₈ O	-	16.503	0.46
Eucalyptol	154.136	C ₁₀ H ₁₈ O	-	10.854	-
Methyl cinnamate	162.068	C ₁₀ H ₁₈ O	-	7.527	-
Camphene	136.125	C ₁₀ H ₁₆	-	4.302	-
β-trans-Ocimene	134.218	C ₁₀ H ₁₆	-	2.451	-
Linalool	154.249	C ₁₀ H ₁₈ O	-	1.966	-
endo-Borneol	154.136	C ₁₀ H ₁₈ O	-	0.994	-
Camphene hydrate	154.136	C ₁₀ H ₁₈ O	-	0.954	-
Terpinolene	136.125	C ₁₀ H ₁₆	-	0.315	9.93
β-Phellandrene	136.125	C ₁₀ H ₁₆	-	-	18.85
γ-Terpinene	136.125	C ₁₀ H ₁₆	-	-	4.54
β-Pinene	136.125	C ₁₀ H ₁₆	-	-	4.42
β-Thujene	136.125	C ₁₀ H ₁₆	-	-	2.57
p-Menth-2-en-1-ol	154.136	C ₁₀ H ₁₈ O	-	-	2.08
Scopoletin	192.042	C ₁₀ H ₁₈ O	-	-	1.85
β-Cedrene	204.188	C ₁₅ H ₂₄	-	-	1.65
α-Thujene	136.125	C ₁₀ H ₁₆	-	-	1.47
α-Terpineol	154.136	C ₁₀ H ₁₈ O	-	-	1.37
2-Cyclohexen-1-ol, 3-methyl-6-(1-methyl ethyl)-, cis-	136.125	C ₁₀ H ₁₈ O	-	-	0.78
cis-Piperitol	154.136	C ₁₀ H ₁₈ O	-	-	1.20
cis-Thujane-4-ol	154.136	C ₁₀ H ₁₈ O	-	-	1.13
2-Carene	136.125	C ₁₀ H ₁₆	-	-	0.48
α-Phellandrene	136.125	C ₁₀ H ₁₆	-	-	0.42
Cinnamyl acetate	176.212	C ₁₁ H ₁₂ O ₂	-	-	0.35

All data showed average values derived from three separate injection, analyzed under identical GC-MS conditions.

The results showed differences in the major components between the essential oils of *C. mangga*, *B. rotunda*, and *Z. purpureum*. The main chemical components of *C. mangga* essential oil were β-

myrcene (29.402%), L-β-pinene (20.916%), cineole (10.056%). It differed from the study conducted in Kuantan, Malaysia, which showed the main chemical components were caryophyllene oxide

(18.71%) and caryophyllene (12.69%) [16]. On the other hand, *B. rotunda* essential oil contained 17 compounds, with the major components being camphor (28.433%), ocimene (16.689%), and geraniol (16.503%). These results were not much different from previous research in Thailand, where by β -ocimene was the most significant component of *B. rotunda* essential oil, followed by geraniol and camphor compounds [29].

The chemical components contained in *Z. purpureum* essential oil were terpinene-4-ol (30.30%), β -phellandrene (18.85%), terpinolene (7.27%). Previous studies also reported terpinene-4-ol as the main constituent (30). The three samples mainly consisted of monoterpenes rather than sesquiterpenes. The variation of chemical compositions was significantly affected by the varieties of plants, agroclimatic conditions (climate, seasonality, and geography), stage

of maturity, adaptive metabolism of the plant, distillation conditions, and part of the plant used [16].

Antibacterial activity

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values are shown in table 5 and fig. 3, 4. The determination of the MIC value is determined by the lowest concentration where no color change occurs after the addition of MTT. The purple color change indicates bacterial growth in the well, as the tetrazolium salt is reduced to purple formazan crystals by the succinate dehydrogenase enzyme produced by the mitochondria of viable cells. Meanwhile, the MBC value is based on the lowest concentration where no bacterial growth is observed after each well is swabbed with a sterile cotton bud and inoculated onto Nutrient agar for 24 h at 37 °C.

Table 5: Antibacterial activity of *C. mangga*, *Boesenbergia rotunda* L. Mansf, and *Z. purpureum* roscoe essential oil

Sample	Gram-positive (<i>S. aureus</i>)		Gram-negative (<i>E. coli</i>)	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>C. mangga</i>	100	100	200	200
<i>B. rotunda</i>	100	100	100	100
<i>Z. purpureum</i>	25	50	12,5	18,75
Oxfloxacin	0.0014	0.0014	0.00072	0.00072

All data show MIC and MBC values as the mean of four repetitions with ofloxacin positive control and DMSO negative control.

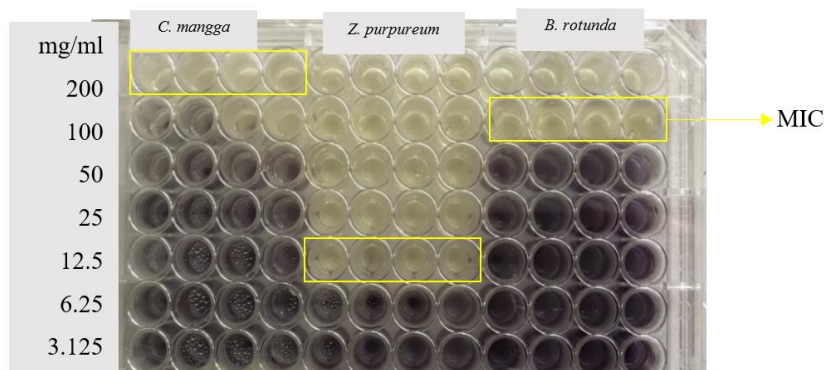


Fig. 3: MIC for essential oils activity against *E. coli* bacteria with microdilution technique. MIC *C. mangga*: 200 mg/ml, *B. rotunda* 100 mg/ml, *Z. purpureum* mg/ml

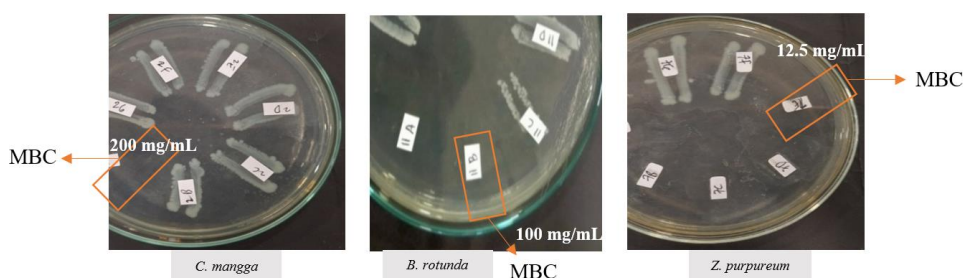


Fig. 4: MBC for essential oils activity against *E. coli* bacteria with streak plate method MBC *C. mangga*: 200 mg/ml, *B. rotunda* 100 mg/ml, *Z. purpureum* mg/ml

The essential oils of *C. mangga* and *B. rotunda* showed lower MIC and MBC values against Gram-positive bacteria. The MIC value is the lowest concentration of the oil that inhibits the visible growth of a microorganism, while the MBC value is the lowest concentration that kills the microorganism. On the other hand, *Z. purpureum* essential oils had a lower MIC value against Gram-negative bacteria. The antibacterial activity was attributed to the presence of oxygenated monoterpene compounds [31].

Z. purpureum essential oil contained significant amounts of terpinene-4-ol, which is known for its antibacterial properties due to

the presence of a hydroxyl moiety. This compound has hydrophobic properties that interfere with bacterial cell growth by reducing intracellular ATP reserves, lowering bacterial membrane potential, and decreasing intracellular pH. Additionally, terpenes in the oil can damage bacterial cell walls through their lipophilic groups. *B. rotunda* essential oil contains camphor, which has a ketone moiety that can penetrate cell membranes and disrupt normal cell function [29, 32]. Studies have shown that oxygenated terpenoids, such as terpene alcohols and phenolics, exhibit better antibacterial activity than carbonyl constituents. Another report ranked the antimicrobial activity

of different compounds as follows: phenols>aldehydes>ketones>alcohols>esters>hydrocarbon groups [33].

Several factors, including genotype, environment, seasonal variations, soil composition, plant parts, and harvest time, can influence the diversity of the chemical composition of essential oils. The plant's geographical origin, the plant part used, and the extraction method are three key factors that significantly affect the chemical and concentration composition of essential oils [34]. In previous research, the antibacterial activity of *C. mangga* essential oil was more effective, with the MIC against Gram-positive bacteria *Staphylococcus aureus* at a concentration of 1.2 µl**/ml. This difference in activity is attributed to the varying composition of *C. mangga* essential oil, where the major components are β-Myrcene (29.402%) and L-β-Pinene (20.916%) [35]. This study concentrated on testing antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, which are common pathogens. However, this study is limited as it only includes these two bacterial species. Testing against a broader range of bacteria, especially antibiotic-resistant strains, could provide a more comprehensive understanding of the antibacterial potential of these compounds. Therefore, future research should include testing on various pathogenic bacteria, including resistant strains, to explore the broader applications of these compounds in the development of effective antibacterial therapies.

Table 7: Anticollagenase activity of *C. mangga*, *Boesenbergia rotunda* L. Mansf, and *Z. purpureum* roscoe essential oil

EO of sample	Concentration (mcg/ml)	% Inhibition±SD
<i>C. mangga</i>	200	15.456±0.182
	100	14.664±0.554
	50	13.434±0.191
	25	12.390±0.588
	12.5	12.123±0.816
<i>B. rotunda</i>	200	46.278±0.138
	100	17.170±0.539
	50	15.343±0.982
	25	17.878±0.606
	12.5	17.808±0.855
<i>Z. purpureum</i>	200	23.990±0.927
	100	20.067±0.410
	50	19.056±0.535
	25	17.098±0.630
	12.5	12.886±1.681
1,10-Phenanthroline	180	70.054±0.557

All data showed as mean±SD (n=3); where n is the number of observations and 1,10-Phenanthroline as positive control.

Based on table 6, the IC₅₀ values of *C. mangga* essential oil, *B. rotunda*, and *Z. purpureum* in inhibiting tyrosinase enzyme were 1.283, 2.897, and 12.030 mg/ml, respectively. Meanwhile, the positive control, kojic acid, obtained an IC₅₀ value of 0.097 mg/ml. Kojic acid had better activity than *C. mangga* essential oil, *B. rotunda* essential oil, and *Z. purpureum* essential oil.

Based on table 4, *C. mangga* essential oil consisted of β-myrcene as the largest component (29.402%). β-myrcene was reported to have protective effects on UVB-irradiated human skin fibroblasts. It has potential protection effects against photoaging by reducing the production of reactive oxygen species (ROS), MMP-1, MMP-3, and IL-6 and increasing the TGF-β1 and type I procollagen secretion. In addition to its antioxidative properties, β-myrcene may also inhibit tyrosinase activity. The reduction in ROS production by β-myrcene could lead to the inhibition of tyrosinase, as oxidative stress is known to activate this enzyme, contributing to melanin production. This suggests that β-myrcene could be beneficial in preventing hyperpigmentation and improving skin tone. Thus, myrcene is potentially an active constituent of skin care products aimed at both photoaging and pigmentation issues [37].

In the anti-collagenase assay, the positive control 1,10-Phenanthroline was used due to its proven ability to inhibit the collagenase enzyme. Phenanthroline structure mimics collagenase enzyme, which contains a tricyclic aromatic hydrocarbon. Collagenase degrades collagen in the skin, contributing to wrinkle

Anti-tyrosinase and anti-collagenase activity

Anti-tyrosinase activity of the of *C. mangga*, *Z. purpureum*, and *B. rotunda* essential oils and kojic acid compounds as positive controls displays in table 6. The IC₅₀ value shows the concentration of a sample that can inhibit 50% of tyrosinase and collagenase activity. The smaller the IC₅₀ value means the higher the inhibitory activity against the tyrosinase and collagenase enzymes [36].

Table 6: Anti-tyrosinase activity of *C. mangga*, *Boesenbergia rotunda* L. Mansf, and *Z. purpureum* roscoe essential oil

Sample	Anti-tyrosinase IC ₅₀ (mg/ml)
<i>C. mangga</i>	1.283±0,077
<i>B. rotunda</i>	2.897±0,135
<i>Z. purpureum</i>	12.030±0,567
Kojic acid	0.097±0,002

All data showed as mean±SD (n=3); where n is the number of observations and kojic acid as positive control, The anti collagenase activity of *C. mangga*, *Z. purpureum*, *B. rotunda*, and 1,10-Phenanthroline essential oils can be seen in table 7.

formation and the aging process. Therefore, phenanthroline that inhibits this enzyme could potentially delay wrinkle formation and the visible signs of aging [34]. However, the results showed that the inhibition by *C. mangga*, *B. rotunda*, and *Z. purpureum* essential oils was relatively weak compared to the positive control. The essential oils contain terpenoid components made of isoprene (C₅H₈) that may not effectively interact with the enzyme's active sites.

CONCLUSION

The essential oils derived from different rhizomes contain varying chemical compounds, although some components were similar. The three essential oils showed weak antibacterial activity, but the essential oil from *Z. purpureum* showed stronger antibacterial activity than those from *B. rotunda* and *C. mangga*. Regarding anti-tyrosinase activity, the essential oil from *C. mangga* performed better than those from *B. rotunda* and *Z. purpureum*. However, when tested for anti-collagenase activity, none of the three essential oils showed any inhibitory activity against collagenase enzymes.

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AUTHORS CONTRIBUTIONS

Suryati Syafri: Methodology, Writing-Original Draft, Writing-Review and Editing; Faincornelis Dehotman Zai: Methodology, Writing-Original Draft, Nabilah Nur Hanifah, Methodology, Writing-Original Draft Amanda Zulfika Putri: Methodology, Writing-Original Draft; Yohanes Allen: Supervision, Review and Editing; Dachriyanus: Supervision, Resources, Funding acquisition, Review and Editing.

CONFLICT OF INTERESTS

Authors state no conflict of interest

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