BIODIVERSITAS
Volume 26, Number 10, October 2025
Pages: 5132-5144

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d261028

Pharmacognostic authentication and bioactivity of polyherbal essential oils from red ginger, turmeric, and aromatic ginger

ALWANI HAMAD[1,♥], RETNO WAHYUNINGRUM[2], DIANA HASRI PUSPARINI[2], DINDA FARIDAENI[2], ELVIA MITA ISABILA[2], DWI HARTANTI[2,♥♥]

[1]Department of Chemical Engineering, Faculty of Engineering and Sciences, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Banyumas 53182, Central Java, Indonesia. Tel.: +62-281-636751, Fax.: +62-281-637239, ♥email: alwanihamad@ump.ac.id
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Banyumas 53182, Central Java, Indonesia. Tel.: +62-281-636751, Fax.: +62-281-637239, ♥♥email: dwihartanti@ump.ac.id

Manuscript received: 24 May 2025. Revision accepted: 13 October 2025.

Abstract. *Hamad A, Wahyuningrum R, Pusparini DH, Faridaeni D, Isabila EM, Hartanti D. 2025. Pharmacognostic authentication and bioactivity of polyherbal essential oils from red ginger, turmeric, and aromatic ginger. Biodiversitas 26: 5132-5144.* People in Baturraden, Indonesia, use rhizomes of red ginger (*Zingiber officinale* var. *rubrum*), turmeric (*Curcuma longa*), and aromatic ginger (*Kaempferia galanga*) to make polyherbal formulations for managing *masuk angin* symptoms. This study aimed to verify the identity of the crude drug, determine the antioxidant properties of the formulations, and evaluate the chemical constituents and antimicrobial activity of polyherbal essential oils to assess the effectiveness for curing *masuk angin* symptoms. The identity of the red ginger, turmeric, and aromatic ginger crude drugs was conducted by following the pharmacopoeial method. The crude drugs were prepared into seven formulations, designated as Formula 1 through Formula 7. The antioxidant properties were evaluated using the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method, and the Total Phenolic Content (TPC) was determined using UV-Vis spectrophotometry. The antimicrobial activity was determined using the microdilution method, with the percentage of inhibition as a parameter. The polyherbal formulations exerted different DPPH scavenging activity and TPC, with the highest values observed in Formula 1[red ginger only, 178.01±3.88 mmol Trolox Equivalent (TE)/g] and Formula 2 [turmeric only, 9.60±0.28 mg Gallic Acid Equivalent (GAE)/g], respectively. Each polyherbal essential oil contained a different constituent profile. Polyherbal essential oils showed moderate antimicrobial activity against Multidrug-Resistant (MDR) *Streptococcus aureus* and *Candida albicans,* with more profound antifungal activity than antimicrobial activity. Formula 3 demonstrated the highest overall antimicrobial activity, with percentages of inhibition against *Streptococcus aureus* and *Candida albicans* were 15.75±1.32% and 71.67±3.39%, respectively. The findings confirmed the identity of the crude drugs, highlighting the potential of a local polyherbal formulation, particularly Formula 3, for its antioxidant and moderate antimicrobial activities associated with *masuk angin* symptoms.

Keywords: *Curcuma longa*, essential oils, *Kaempferia galanga*, polyherbal formulation, *Zingiber officinale* var. *rubrum*

INTRODUCTION

Zingiberaceae plants, particularly red ginger (*Zingiber officinale* var. *rubrum* Theilade), turmeric (*Curcuma longa* L.), and aromatic ginger (*Kaempferia galanga* L.), are widely recognized in Indonesian traditional herbal medicine for their therapeutic properties (Widyowati and Agil 2018). Red ginger rhizomes contain phenolics, essential oils, terpenoids, and diarylheptanoids with analgesic, antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant effects (Wijayanti et al. 2018; Zhang et al. 2022). Turmeric is rich in curcuminoids, flavonoids, and polysaccharides responsible for anti-inflammatory, antimicrobial, antioxidant, and metabolic benefits (Ahmad et al. 2020). Aromatic ginger's essential oils—dominated by ethyl p-methoxycinnamate, methyl cinnamate, and 1,8-cineole—along with flavonoids and terpenoids, contribute to its antimicrobial, analgesic, anti-asthmatic, and antioxidant properties (Kumar 2020).

In Baturraden, Central Java, Indonesia, a polyherbal formulation composed of red ginger, turmeric, and aromatic ginger rhizomes has been traditionally used to manage *masuk angin* (Utaminingrum et al. 2021). Being a mixture of several medicinal plants, polyherbal formulations are expected to exert synergistic potential. It refers to the idea that the combined effect of the formulation is greater than the sum of the individual component effects. The multiple bioactives in the polyherbal formulations might target various pathways involved in disease development. They may also modify the pharmacokinetics of the main active compounds, resulting in improved efficacy or reduced toxicity (Heinrich et al. 2023). The application of polyherbal formulations in traditional use is justified by a holistic understanding of health, empirical clinical practice, and a deliberate strategy to create effective and safe herbal medicines that work in harmony with the complex systems of the body (Murudkar et al. 2022).

In Javanese ethnomedicine, *masuk angin* refers to a bodily imbalance characterized by symptoms like abdominal bloating, muscle aches, chills, fatigue, mild fever, and nausea. While not formally recognized in conventional medicine, *masuk angin* shares symptoms with mild gastrointestinal issues, upper respiratory infections, and psychosomatic stress (Triratnawati 2011; Prayoga and Pradipto 2014). This overlap makes *masuk angin* remedies

a suitable model for evaluating effectiveness. A recent bioinformatics study found that herbal medicines should target primary symptoms and offer supporting effects, such as anti-inflammatory, antioxidant, immunomodulatory, analgesic and antimicrobial properties, to help address fatigue and debility (Afendi et al. 2016). Therefore, evaluating a polyherbal formulation for *masuk angin* can include testing its analgesic, antipyretic, and anti-inflammatory properties, along with its antimicrobial, antioxidant, and immunomodulatory activities.

Despite its long history of use, scientific validation of the local *masuk angin* polyherbal formulation remains insufficient. To address this gap, the present study evaluate the antioxidant properties, essential oil chemical profile, and antimicrobial activity against Multidrug-Resistant (MDR) *Staphylococcus aureus* and *Candida albicans* from formulations with varying ratios of red ginger, turmeric, and aromatic ginger. The identity of these crude drugs was confirmed by macroscopic and microscopic morphology, along with Thin-Layer Chromatography (TLC) profiling, following Indonesian Herbal Pharmacopoeia (IHP) standards (Indonesian MoH 2017). Phenolic content and antioxidant capacity were assessed using Total Phenolic Content (TPC) and DPPH assays, respectively. The antioxidant effects of these rhizomes are attributed to their high phenolic content (Ahmad et al. 2020; Kumar 2020; Zhang et al. 2022).

The Indonesian MoH (2017) identifies volatile oil as the standard chemical marker for red ginger, turmeric, and aromatic ginger rhizomes. The essential oil profiles of these plants are well-documented (Rinanda et al. 2018; Wang et al. 2023; Orellana-Paucar 2024), but there is limited research on the distillation of combined crude drugs. This study analyzed the chemical composition of essential oils from hydrodistilled formulations containing various ratios of these three plants using Gas Chromatography-Mass Spectrometry (GC-MS). Red ginger, turmeric, and aromatic ginger oils individually show significant antimicrobial properties (Yang and Rahmawati 2022; Muzzazinah et al. 2024), but their combined effects are unexplored. Combining essential oils may enhance antimicrobial activity and serve as potential resistance breakers for Multidrug-Resistant (MDR) microorganisms, offering renewed efficacy for existing antibiotics (Ju et al. 2022). In this study, the antimicrobial activity of the essential oils from red ginger, turmeric, and aromatic ginger, as well as those obtained from crude drug combinations, was evaluated against patient-isolated MDR *Streptococcus aureus* and *Candida albicans*.

MATERIALS AND METHODS

Materials

Crude drugs (red ginger, turmeric, and aromatic ginger rhizomes) were purchased from Hortus Medicus Jamu Research Center, Karanganyar, Central Java, Indonesia. Chemical and reagents [2,2-diphenyl-1-picrylhydrazyl (DPPH), anhydrous sodium sulfate, anisaldehyde-sulfuric acid solution, dimethyl sulfoxide (DMSO), Follin Ciocalteau reagent, and iodine solution], standards (curcuminoids, ethyl p-methoxycinnamate, eugenol, gallic acid, and Trolox), and solvents (acetone, chloroform, ethanol, ethyl acetate, methanol, toluene, and water) were obtained from Sigma-Aldrich (St. Louis, United States). The microbiological media [Mueller-Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB)] were obtained from Oxoid (Hampshire, United Kingdom).

Procedures

Identification of the crude drugs

The crude drugs were authenticated through macroscopic and microscopic observations, as well as their TLC profiles, following the identity fragments and standard chromatograms defined in the IHP (Indonesian MoH 2017). Microscopic observation of the powdered crude drugs was conducted using an Optilab camera (Beijing, China) attached to a light microscope (Olympus, Tokyo, Japan). The mobile phase, reference compound, and detection method for red ginger crude drugs were toluene: acetone (9:1), eugenol, and under visible light after derivatization with anisaldehyde-sulfuric acid, whereas those for turmeric were chloroform: methanol (95:5), curcuminoid, and under UV lamps at 254 nm. Toluene: ethyl acetate (95:5), ethyl p-methoxycinnamate, and UV_{254} were utilized for the aromatic ginger chromatographic profile.

Polyherbal formulation preparation

Red ginger, turmeric, and aromatic ginger powdered crude drugs were homogeneously mixed into seven formulations, i.e., Formulas 1-7 (Table 1).

Evaluation of polyherbal antioxidant activities

The DPPH radical scavenging activity and TPC of each formulation were evaluated using established methods (Hartanti et al. 2023). Extraction was carried out using 70% ethanol via maceration for 2 days. DPPH scavenging activity was reported as mmol Trolox Equivalent (TE)/g extract, using the Trolox concentration-absorbance curve equation: $y = 0.2438x-1.759$; $R^2 = 0.991$. The theoretical DPPH scavenging activity of the crude drug combination-containing formulations was calculated from the weighted values of the single-drug activities, based on their proportions in the mixtures. As previously reported, the interaction effect between the actual and theoretical DPPH scavenging activity was evaluated using the comparison and difference method (Hartanti and Hamad 2023; Yap et al. 2023). TPC was reported as mg Gallic Acid Equivalent (GAE)/g extract, using a gallic acid linear curve equation: $y = 0.0057x + 0.0258$; $R^2 = 0.993$.

Table 1. Preparation of the polyherbal formulations

Formulation	Crude drug's weight ratio (%)		
	Red ginger	Turmeric	Aromatic ginger
Formula 1	100	0	0
Formula 2	0	100	0
Formula 3	0	0	100
Formula 4	33	33	33
Formula 5	50	25	25
Formula 6	25	50	25
Formula 7	25	25	50

Essential oil distillation

Two kg of each formulation of crude drug were hydro-distilled for approximately six hours. The essential oil was added to anhydrous sodium sulfate to remove water traces. The color and scent of the oils were observed, and the distillation yield was calculated. The theoretical yields of Formulas 4-7 were calculated by combining the yield percentage of each single crude drug-containing formula according to the ratio of the mixtures. The relative difference between actual and theoretical yields using an equation previously described (Rahim et al. 2020).

Essential oil's composition analysis

Polyherbal essential oils were analyzed using GC-MS (Shimadzu QP2010 SE, Kyoto, Japan), equipped with an Rtx-5MS GC capillary column (30 m, 0.25 mm ID, 0.25 μm) and Helium as the carrier gas. The column oven was set to 75°C, the injector temperature was set to 175°C, and 8 μL of the sample was injected in split mode with a split ratio of 50:1 The pressure was maintained at 45.9 kPa, with a total flow rate of 43.8 mL/min, a column flow of 0.80 mL/min, a linear velocity of 32.9 cm/s, and a purge flow of 3.0 mL/min. The column temperature was held at 50°C for 5 minutes, then increased gradually to 250°C, held for 10 minutes, and raised to 300°C for 5 minutes. The interface temperature was maintained at 230°C. The mass spectrometer was configured with an ion source temperature of 225°C, solvent cut time of 2.00 minutes, and a detector gain of 1.14 kV + 0.00 kV. The MS scanning frequency ranged from 10 to 500 m/z. Compounds were identified by comparing the mass spectra with the Wiley9.lib database (Hamad et al. 2021). The actual relative concentrations of major compounds and compound groups in a single crude drug-containing Formula were used to calculate the theoretical relative concentration and percentage of those in the combination crude drug-containing formula. The calculations and equations used to determine the theoretical and percentage yield, with the relative concentrations serving as the response instead of the yield (Rahim et al. 2020).

Antimicrobial activity evaluation

The microorganisms, patient-isolated MDR *Staphylococcus aureus* (resistant to benzylpenicillin, oxacillin ciprofloxacin, clindamycin, erythromycin, levofloxacin, and trimethoprim) and *Candida albicans* (resistant to amphotericin B, caspofungin, micafungin, and voriconazole), were obtained from the Department of Parasitology, Faculty of Medicine, Universitas Indonesia (Jakarta, Indonesia). The antimicrobial activity assay was conducted using the microdilution method, with gentamicin and fluconazole as the positive controls and MHB and SDB as negative controls. Cultures of MDR *S. aureus* and *C. albicans* were incubated at 37°C for 24 hours in MHB and SDB, respectively. The final density of each culture was 1×10^8 CFU/mL. Essential oils from each formulation were prepared in ethanol as a cosolvent to a final concentration of 0.1%. The samples were serially diluted in suitable media to concentrations of 250, 125, 62.5, 31.25, 15.63, and 7.81 μL/mL. One hundred μL of microbial culture and 100 μL of extract solution were added to the 96-well microplates. From the control group, 100 μL of microbial culture and 100 μL of MHB or SDB were used for *S. aureus* and *C. albicans,* respectively. The microplates were incubated at 27±1°C for 24 hours, and absorbances were measured using a microplate reader (BioTek Epoch, United States). The percentage inhibition of microbial growth was calculated by comparing microbial growth in the treated groups to that in the control group (Hulankova 2024).

Data analysis

All data were obtained from three replicate experiments. The TPC, DPPH scavenging activity, and % inhibition at 7.81 μL/mL of each formulation oil against each tested microorganism were evaluated using One-way ANOVA and Duncan's test. Correlations between these two antioxidant parameters were assessed using Pearson's correlation coefficient. The actual and theoretical values of the DPPH scavenging activity of Formulas 4-7 were compared using an independent t-test. All statistical analyses were conducted using SPSS ver 26, with significant effects, differences, and correlations assigned at $p<0.05$ (IBM, US).

RESULTS AND DISCUSSION

Pharmacognostic identification of crude drugs

The macroscopic characteristics of the crude drugs (Figure 1) were consistent with the descriptions in the IHP (Indonesian MoH 2017). All diagnostic fragments of red ginger, i.e., starch granules, parenchyma with oil-containing idioblasts, vessels, and fiber in bundles, were observed. Similarly, all six diagnostic fragments of turmeric, i.e., starch granules, parenchyma with curcuminoid-containing idioblasts, vessels, and covering trichomes, were observed (Figure 2). Smaller starch granules, parenchyma, vessels, and periderms of aromatic ginger were also observed. Hence, all required diagnostic fragments listed in the IHP monograph were present. The TLC profiles of red ginger, turmeric, and aromatic ginger were similar to those of IHP (Figure 3). Therefore, their identity had been confirmed based on the morphology and TLC profile.

Antioxidant properties of polyherbal formulations

The proportion of each crude drug in the formulation significantly affected the phenolic compound content ($p<0.05$). The turmeric crude drugs in Formula 2 had the highest TPC, and the aromatic ginger rhizomes (Formula 3) had the lowest TPC. The formulation with a higher fraction of turmeric (Formula 6) and an equal mixture of the three crude drugs (Formula 4) had the highest phenolic compound content among the other formulations (Table 2). The component weight ratio significantly affected the DPPH free radical scavenging activity ($p<0.05$). Red ginger (Formula 1) and aromatic ginger (Formula 2) had the strongest and weakest radical scavenging activities, respectively. Hence, a formulation with a higher ratio of red ginger (Formula 5) exhibited higher DPPH scavenging activity. Furthermore, both prediction methods revealed an antagonist interaction effect in all formulations containing the crude drug mixture (Table 2).

TPC was strongly and significantly correlated with DPPH scavenging activity in formulations of a single plant (Formulas 1-3). However, TPC did not correlate with DPPH scavenging activity in formulations consisting of different ratios of the three components (Figure 4). Hence, the phenolic compounds are likely responsible for the radical scavenging activity of each crude drug.

Antimicrobial activity of polyherbal essential oils

The yield of the formulation of oil distillation ranged from 0.55 to 0.85%, with a color of orangish brown to brown (Table 3). Each formulation contained different chemical constituents (Table 4). A total of 37 compounds were identified in Formula 1, including significant amounts of ar-curcumene, camphene, 1,8-cineole, geranial, neral, β-bisabolene, β-sesquiphellandrene, and zingiberene. The Formula 2, turmeric, consisted of 35 compounds, with β-turmerone, α-turmerone, 1,8-cineole, 1-(3-cyclopentyl propyl)-2,4-dimethyl-benzene, ar-curcumin, and p-cymene as the major ones. However, Ar-turmerone is the primary turmeric oil compound obtained from crude drugs. Formula 3, which only contained aromatic ginger oil, comprises 39 compounds, with the main compounds being 3-carene, ethyl p-methoxycinnamate, β-turmerone, and p-cymene. The compounds detected in Formulas 1, 2, and 3 were α-pinene, camphene, ar-curcumene, zingiberene, β-bisabolene, and β-sesquiphellandrene.

Figure 1. Macroscopic characteristics of: A. Red ginger, B. Turmeric, and C. Aromatic ginger crude drugs

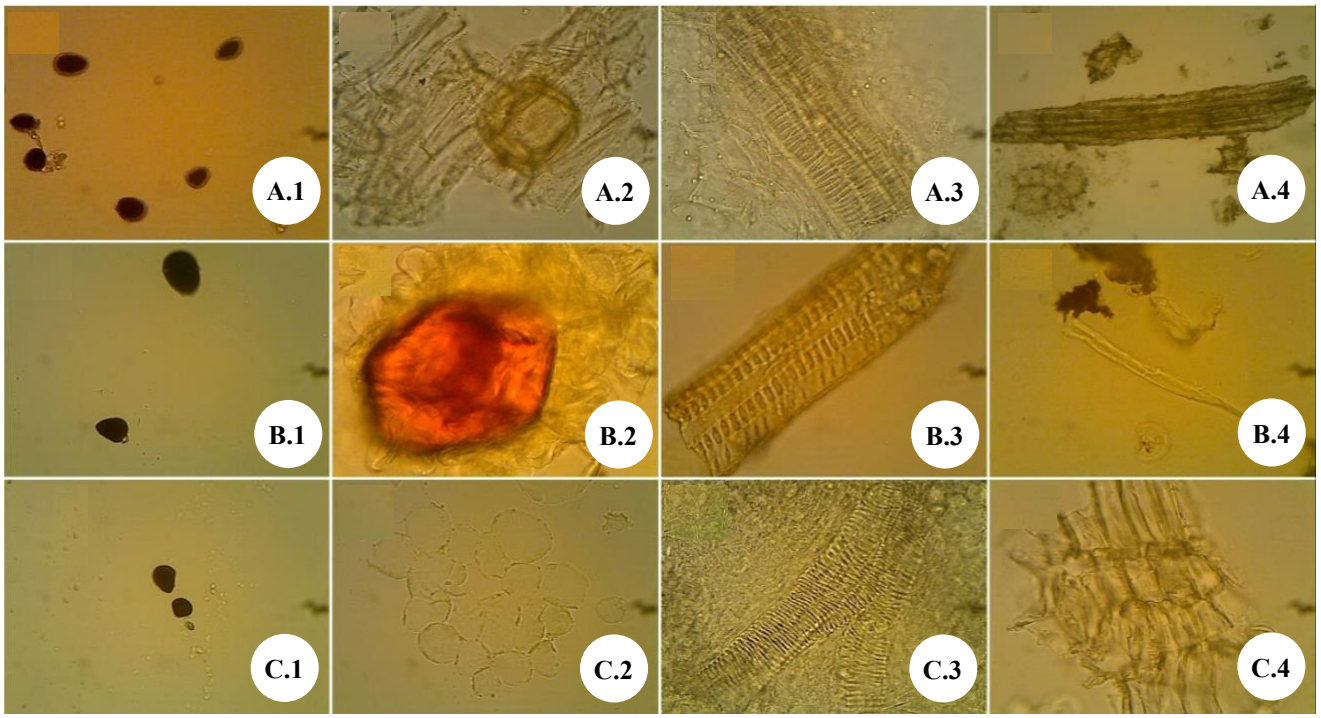

Figure 2. The microscopic characteristics of: A. Red ginger, B. Turmeric, and C. Aromatic ginger crude drugs showing: 1. Starch granules, 2. Parenchyma with idioblast containing oil in red ginger and yellow pigments in turmeric, 3. Vessels, and 4. Fiber bundle in red ginger, covering trichome in turmeric, and periderm in aromatic ginger

Table 2. The TPC and DPPH scavenging activity of the polyherbal formulations (n = 3)

Formulation	DPPH (mmol TE/g)				TPC (mg GAE/g)
	Actual	Theoretical	Difference	Interaction effect	
Formula 1	178.01±3.88^{F}	-	-	-	2.77±0.01^{B}
Formula 2	88.68±2.33^{D}	-	-	-	9.60±0.28^{G}
Formula 3	63.48±0.73^{A}	-	-	-	0.73±0.03^{A}
Formula 4	79.96±0.75^{C}	110.05±0.85*	-75,78	Antagonist	5.17±0.06^{E}
Formula 5	116.34±2.50^{E}	127.04±2.55*	-64,76	Antagonist	4.21±0.04^{D}
Formula 6	73.23±0.50^{B}	104.71±0.52*	-77,82	Antagonist	6.21±0.04^{F}
Formula 7	66.46±0.54^{A}	98.41±0.59*	-79,87	Antagonist	3.90±0.04^{C}

Note: Different letters within the actual DPPH scavenging activity and TPC columns represent statistically different values. *: Indicates that the theoretical values are statistically lower than the actual ones (n = 3)

Table 3. The yield and organoleptic characteristics of the oils

Essential oil	Yield (%)			Color	Scent character
	Actual	Theoretical	Percentage		
Formula 1	0.80	-	-	Orangish brown	A rich, spicy, warm, with a zesty and earthy undertone aroma
Formula 2	0.55	-	-	Orangish brown	A warm, earthy, slightly spicy, with a subtle woody undertone aroma
Formula 3	0.65	-	-	Brown	A fresh, camphoraceous, slightly sweet, with a warm and spicy undertone aroma
Formula 4	0.75	0.67	113	Brown	A mixture of red ginger, turmeric, and aromatic ginger aroma
Formula 5	0.70	0.70	100	Brown	A mixture of red ginger, turmeric, and aromatic ginger aroma with a stronger hint of red ginger
Formula 6	0.80	0.64	125	Orangish brown	A mixture of red ginger, turmeric, and aromatic ginger aroma with a stronger hint of turmeric
Formula 7	0.85	0.66	128	Orangish brown	A mixture of red ginger, turmeric, and aromatic ginger aroma with a stronger hint of aromatic ginger

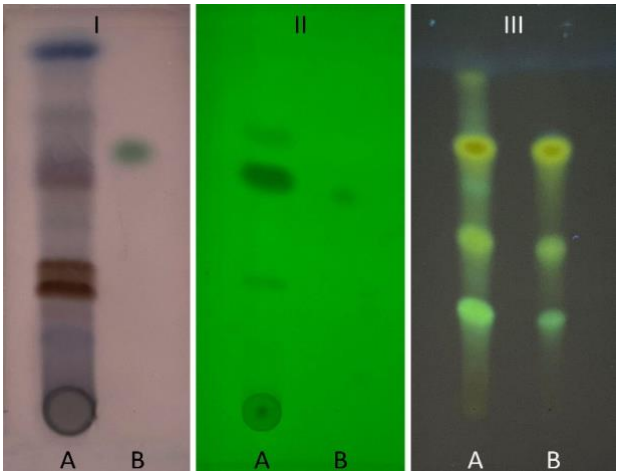

Figure 3. TLC profiles of: I: Red ginger, II: Aromatic ginger, III: Turmeric crude drugs, showing the separation of: A: Extracts, B: Reference compounds

Of the 38 compounds detected in Formula 4 oil, the main ones were 3-carene, β-turmerone, ethyl p-methoxycinnamate, camphene, ar-curcumene, and 1,8-cineole. Formula 5 oil contained 39 compounds with β-turmerone, 1,8-cineole, 3-carene, camphene, ethyl p-methoxycinnamate, ar-curcumene, and geranial as the major constituents. Formula 6 oil consisted of 39 compounds, mainly β-turmerone, ethyl p-methoxycinnamate, 3-carene, ar-curcumene, camphene, p-cymene, α-turmerone, and 1,8-cineole. β-Turmerone, 3-carene, ethyl p-methoxycinnamate, camphene, and 1,8-cineole were the main compounds of Formula 7 oil. Ar-curcumene and camphene of red ginger oil, β-turmerone and α-turmerone of turmeric oil, as well as 3-carene and ethyl p-methoxycinnamate of aromatic ginger oil, were chosen as the representative compounds to compare their relative concentration in polyherbal essential oil. The obtained relative concentrations of the main essential oil constituents in Formulas 4-7 are more or less the sum of their respective concentration fractions in the single crude drug-containing formulations with a concentration percentage of 80-120%, except for α-turmerone, camphene, 3-carene, and ethyl p-methoxycinnamate in Formula 4; camphene, 3-carene, and ethyl p-methoxycinnamate in Formulas 5 and 6, as well as camphene and ethyl p-methoxycinnamate in Formula 7. Formulas 1 and 2 contained a higher concentration of hydrocarbons than the oxygenated compounds, whereas in Formula 4, both compounds were comparable in concentration. In contrast, Formulas 3, 5, 6, and 7 were dominated by oxygenated compounds (Table 5). Hence, individual crude drug distillation followed by combining the obtained oils or combining crude drugs first and then distilling them, is likely to generate oils with similar major constituents.

All formulation oils inhibited MDR *S. aureus* and MDR *C. albicans*. They exhibited microbial growth inhibitory activity at 7.81 µL/mL, indicating moderate antimicrobial activity against both microorganisms. At a concentration of 7.81 µL/mL, MDR *S. aureus* was more susceptible to the

oils than MDR *C. albicans*. The oils of Formulas 3, 4, 6, and 7 exhibited stronger antibacterial activity than the oils of the other formulations, whereas the oils in Formulas 2 and 3 demonstrated higher antifungal activity. Overall, Formula 3, which contained aromatic ginger oil, showed the best antimicrobial activity (Table 6, Figure 5).

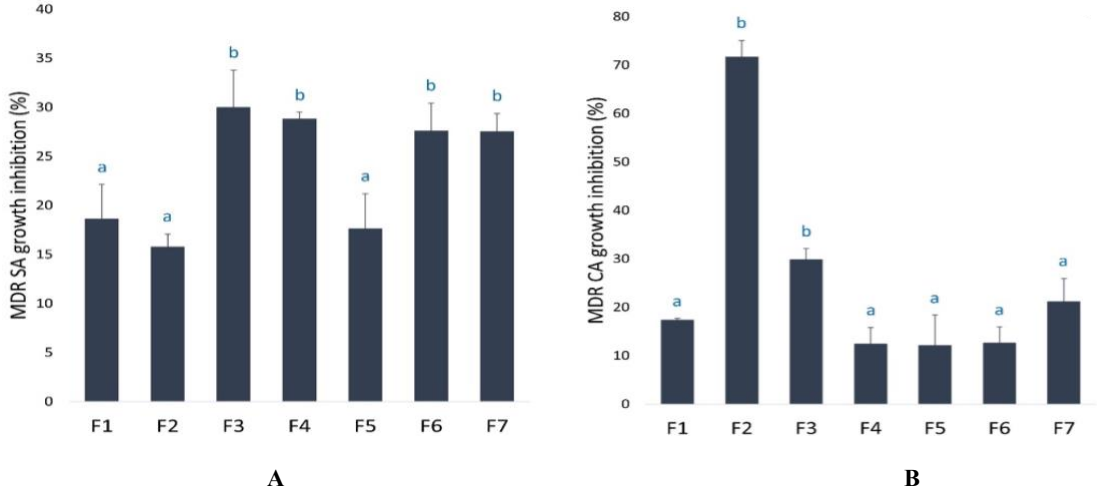

Figure 4. The A. MDR *Staphylococcus aureus*, and B. MDR *Candida albicans* growth inhibition at the 7.81% concentration (n = 3). F1: Formula 1, F2: Formula 2, F3: Formula 3, F4: Formula 4, F5: Formula 5, F6: Formula 6, F7: Formula 7

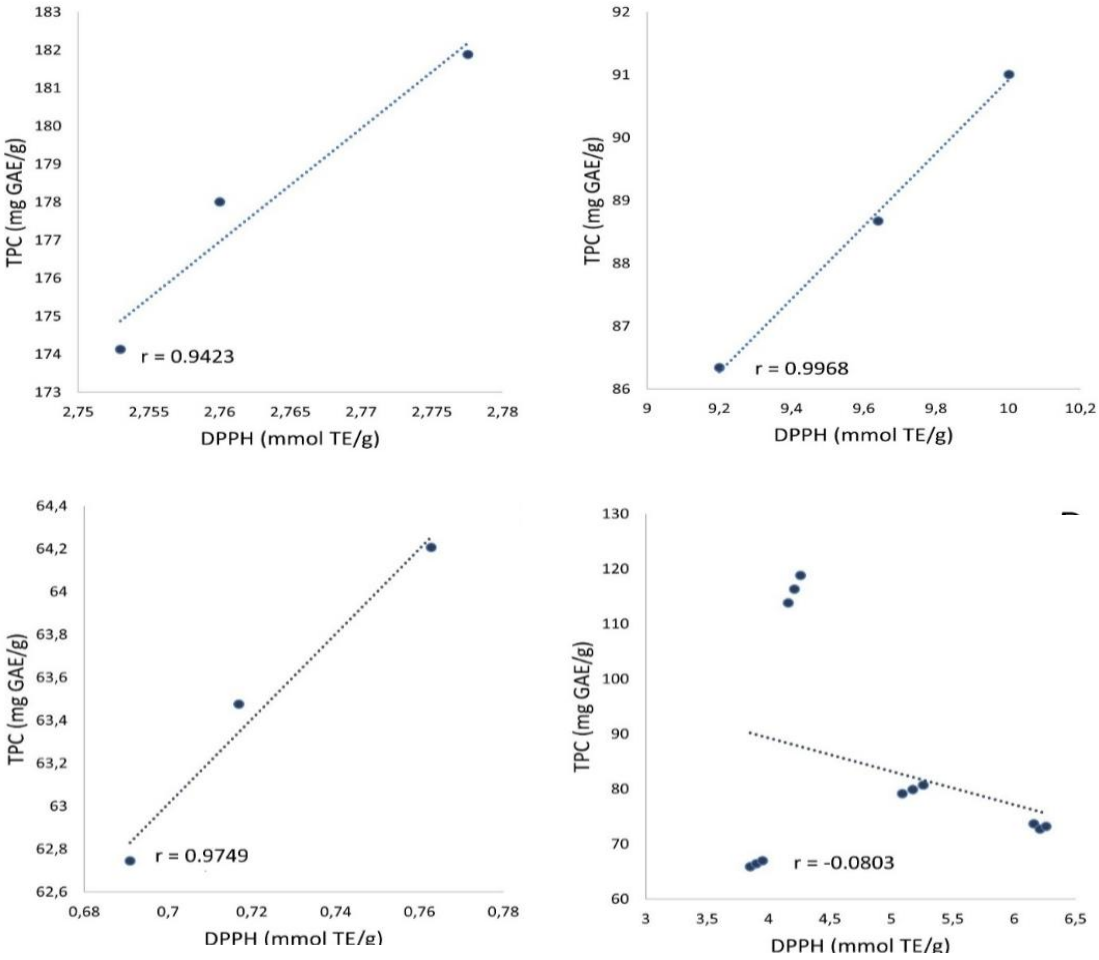

Figure 5. Correlation between DPPH scavenging activity and TPC of: A. Formula 1, B. Formula 2, C. Formula 3, and D. Formula 4-7 (n = 3 per formulation)

Table 4. Chemical composition of polyherbal formulation oils

RT (min)	Compound	% Area in						
		F1	F2	F3	F4	F5	F6	F7
3.210	Tricyclene	0.24	-	0.08	0.14	0.17	0.11	0.14
3.269	α-Thujene	-	-	0.29	0.11	-	0.06	0.14
3.357	α-Pinene	2.38	0.36	2.57	2.50	2.46	1.95	2.48
3.621	Camphene	11.23	0.17	3.00	7.75	8.65	6.69	7.36
4.011	3,7,7-Trimethyl-1,3,5-cycloheptatriene	-	-	0.26	-	-	-	0.14
4.070	β-Pinene	0.26	-	0.61	-	-	-	0.50
4.071	Linalyl acetate	-	-	-	0.52	0.46	0.25	-
4.454	β-Myrcene	0.89	-	0.67	0.36	0.74	0.53	0.70
4.590	trans-2-Caren-4-ol	-	0.02	-	-	-	-	-
4.669	α-Phellandrene	-	2.33	-	1.03	0.45	1.14	0.93
4.770	Sabinene	-	0.46	-	-	-	-	-
4.848	3-Carene	-	-	26.50	17.27	9.67	9.86	15.78
4.679	(E)-β -ocimene	-	-	-	0.10	-	-	-
5.080	α-Terpinene	-	0.06	-		-	-	-
5.210	p-Cymene	-	5.02	8.93	2.28	-	6.54	2.91
5.269	Limonene	-	-	-	2.46	-	-	2.05
5.336	1,8-Cineole	9.50	6.62	-	5.38	9.73	6.44	5.63
6.935	α-Terpinolene	-	-	-	-	0.15	-	-
7.066	Berbenone	0.34	-	0.09	-	-	-	-
7.095	Limonene oxide	-	-	0.09	-	-	-	0.05
7.107	Verbenol	-	-	-	-	0.13	-	-
7.122	Linalool	0.70	-	-	-	0.31	-	-
7220	Isobornyl methyl ether	0.38	-	-	-	-	-	-
8.063	Camphor	0.18	-	-	-	-	-	-
8.164	Camphene hydrate	0.11	-	-	-	-	-	-
8.565	Borneol	4.26	-	0.83	1.77	2.53	1.29	2.11
8.745	p-Mentha-1,5-dien-8-ol	-	-	0.24	-	-	-	-
8.921	4-Terpineol	-	-	0.39	-	0.35	-	-
9.194	α-Terpineol	0.43	-	-	-	-	-	-
9.375	Terpinyl acetate	-	-	0.05	-	-	-	-
9.600	p-Cymen-8-ol	0.14	-	-	-	-	-	0.13
9.671	Eucarvone	-	-	0.11	-	-	-	-
9.864	β-Citronellol	1.12	-	-	0.72	0.48	0.23	0.28
10.063	Neral	8.95	-	-	2.32	4.95	3.50	4.18
10.366	Geranial	9.09	-	-	3.01	7.47	2.38	2.59
10.413	Geraniol	3.30	-	-	0.49	1.20	0.55	0.66
10.689	Bornyl acetate	3.44	-	0.04	-	-	1.82	1.95
10.926	2-Undecanone	0.71	-	-	-	0.10	-	-
11.948	(+)-Cyclosativene	0.16	-	-	-	-	-	-
12.098	α-Copaene	0.21	-	-	-	0.10	-	-
12.380	β-Elemene	-	-	0.09	0.11	0.19	0.08	0.08
12.486	α-Gurjunene	-	-	0.36	0.50	0.49	0.10	0.33
12.803	Caryophyllene	-	0.24	0.23	0.14	0.10	0.12	0.09
12.984	Ylangene	0.19	-	1.25	-	-	-	-
13.185	Ar-curcumene	11.90	5.45	3.05	5.94	7.53	6.81	4.33
13.325	β-Selinene	-	-	0.13	-	-	-	-
13.406	Humulen	-	0.18	-	-	-	-	-
13.415	β-Chamigrene	-	-	0.10	-	-	-	-
13.417	Alloaromadendrene	0.25	-	-	-	0.12	-	-
13.485	β-Cedrene	-	-	0.06	-	-	-	-
13.901	Zingiberene	7.36	1.27	1.57	2.41	2.25	2.57	1.94
13.977	γ-Cadinene	-	-	0.55	0.26	-	0.58	0.26
14.069	β-Bisabolene	7.93	0.90	1.09	1.53	3.08	2.01	1.19
14.273	β -Sesquiphellandrene	7.69	1.54	1.54	1.77	3.04	2.51	1.61
14.422	α-Santalol	-	0.25	-	-	-	-	-
14.485	Valencene	-	-	0.61	-	-	-	-
14.647	Spathulenol	0.14	-	-	0.07	0.16	-	-
14.665	1-Methyl-4-[2-(1-methylethylidene) cyclopropyl]benzene	-	-	0.27	-	-	-	-
14.778	Germacrene B	-	-	1.77	0.59	0.68	0.49	0.61
14.785	1-Methyl-4-methylene-2-(2-methyl-1-propenyl)-1-vinylcycloheptane	0.50	-	-	-	-	-	-
14.879	2,5,9-Trimethylcycloundeca-4,8-dienone	-	0.24	-	-	-	-	-
14.925	Nerolidol	0.24	-	-	-	0.93	-	-
14.935	trans-Carveol	-	0.22	-	-	-	-	-

15.080	Ar-turmerone	-	0.20	-	-	-	0.07	-
15.175	3-Chloro-8-phenyl-menthane	-	4.36	-	-	-	2.17	-
14.805	Caryophyllene oxide	-	0.51	1.56	-	-	-	-
14.147	Dihydrocurcumene	-	0.94	-	1.00	1.70	-	1.20
15.279	α-Bergamotene	0.93	0.07	-	-	2.17	-	-
15.325	4-(2-Butyl)toluene	-		-	0.29	-	-	0.29
15.487	1-(3-cyclopentylpropyl)-2,4-dimethyl-)-benzene	-	5.74	-	-	-	-	-
15.527	α-Cedrene	-	-	-	1.33	-	-	2.06
15.545	Verbenyl acetate	-	-	2.15	-	-	-	-
15.563	Zingiberenol	1.92	-	-	-	-	-	-
15.670	β-Patchoulene	-	-	-	0.15	-	-	-
15.670	α-Longipinene	-	-	-	-	-	0.22	-
15.695	10-epi-γ-eudesmol	0.36	-	-	-	-	-	-
15.781	α-Bisabolol	-		-	-	-	0.55	-
15.787	1-[2-Methyl-2-(4-methyl-3-pentenyl)cyclopropyl]ethanol	-	0.79	-	0.17	-	-	-
15.880	Octahydro-2-methyl-4,2,8-ethanylylidene-2H-1-benzopyran	-	2.63	-	-	0.64	1.09	-
15.889	3,7-Dimethyl-1-(2,5-xylyl)-octane	-		-	-	-	-	0.73
16.021	trans-Longipinocarveol	-	0.98	-	-	0.28	-	0.25
16.055	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane	-	-	-	-	-	0.26	-
16.130	Di-epi-alpha-cedrene-(I)	-	-	-	0.19	-	0.40	-
16.131	Levoverbenone	-	0.29	-	-	-	-	-
16.250	β-Eudesmol	1.02	-	-	-	0.58	-	0.33
16.302	β-Turmerone	-	36.82	14.76	16.8	13.36	19.76	16.25
16.525	Farnesol 1	-	-	-	-	-	0.33	-
16.613	Dehydrolinalool	0.71	-	-	-	-	-	0.43
16.631	1,4-Diphenyl-1-pentanone	-	0.06	-	-	-	-	-
16.734	Dehydronerolidol	0.83	-	-	-	-	-	-
16.771	α-Turmerone	-	13.94	3.88	4.00	4.10	6.48	4.41
16.870	4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-2(1h)-naphthalenone	-	1.65	-	-	-	-	-
17.175	Gossonorol	-	0.23	-	-	-	-	-
17.245	3,4,5,6-Tetramethyl-2,5-octadiene	-	1.42	-	0.19	-	-	-
17.266	Cyclohexene-1-one	-	-	-	-	0.12	-	-
17.531	Ethyl p-methoxycinnamate	-	-	19.96	14.33	8.39	9.90	12.86
17.384	Megastigma-5,8(Z)-dien-4-one	-	-	0.26	-	-	-	-
17.536	2,3,3a,4,7,7a-Hexahydro-2,2,4,4,7,7-hexamethyl-1H-indene	-	3.71	-	-	-	-	-
17.800	2-Cyclohexyl-2-isobutylmalononitrile	-	-	-	-	-	0.04	-
17.835	2-Nitro-2-propenyl-cyclohexane	-	0.31	-	-	-	-	-
18.014	Spiro[bicyclo[3.3.0]octan-6-one-3-cyclopropane]	-	-	-	-	-	0.05	-
18.105	2-Cyclopropylidene-1,7,7-trimethylbicyclo[2.2.1]heptane	-	-	-	0.05	-	-	-
18.965	Methyl palmitate	-	-	0.02	-	-	-	-

Note: F1: Formula 1, F2: Formula 2, F3: Formula 3, F4: Formula 4, F5: Formula 5, F6: Formula 6, F7: Formula 7

Table 5. Comparison of the selected compounds in the polyherbal essential oils

Compounds	Relative concentration (%)														
	Formula 1	Formula 2	Formula 3	Formula 4			Formula 5			Formula 6			Formula 7		
				A	T	%	A	T	%	A	T	%	A	T	%
Ar-curcumene	11.90	5.45	3.05	5.94	6.80	87	7.53	8.08	93	6.81	6.46	105	4.33	5.86	74
Camphene	11.23	0.17	3.00	7.75	4.80	161	8.65	6.41	135	6.69	3.64	184	7.36	4.35	169
α-Turmerone	-	13.94	3.88	4.00	5.94	67	4.10	4.46	92	6.48	7.94	82	4.41	5.43	81
β-Turmerone	-	36.82	14.76	16.80	17.19	98	13.36	12.90	104	19.76	22.10	89	16.25	16.59	98
3-Carene	-	-	26.50	17.27	8.83	196	9.67	6.63	146	9.86	6.63	149	15.78	13.25	119
Ethyl p-methoxycinnamate	-	-	19.96	14.33	6.65	215	8.39	4.99	168	9.90	4.99	198	12.86	9.98	129
Alcohols	15.28	4.14	1.46	4.46	6.96	64	6.95	9.04	77	2.95	6.26	47	20.66	5.59	370
Aldehydes	18.04	0.29	-	3.08	6.11	50	12.42	9.09	137	5.88	4.66	126	47.85	45.82	104
Esters	3.44	0.00	22.22	14.85	8.55	174	8.85	7.28	122	11.97	6.42	187	5.75	11.97	48
Ethers	9.88	9.76	1.65	5.38	7.10	76	10.37	7.79	133	7.86	7.76	101	14.81	5.74	258
Ketones	1.23	51.26	19.10	20.80	23.86	87	17.68	18.21	97	26.36	30.71	86	4.19	22.67	18
Oxygenated compounds	47.88	65.47	44.42	49.55	52.59	94	56.26	51.41	109	55.02	55.81	99	93.23	50.55	184
Hydrocarbons	52.12	34.53	55.58	50.45	47.41	106	43.74	48.59	90	44.98	44.19	102	6.77	49.45	14

Note: A: Actual value, T: Theoretical value

Table 6. The antimicrobial activity of the polyherbal formulation oil against MDR *Streptococcus aureus* and MDR *Candida albicans* (n=3)

Essential oil	Percentage of inhibition (%) at a concentration of					
	250 µL/mL	125 µL/mL	62.50 µL/mL	31.25 µL/mL	15.62 µL/mL	7.81 µL/mL
MDR *Streptococcus aureus*						
Formula 1	20.93±1.66	20.18±2.22	34.34±16.89	25.21±2.47	23.53±1.92	18.61±6.56
Formula 2	26.98±6.42	14.50±4.04	13.67±4.39	14.65±6.19	15.78±6.15	15.75±1.32
Formula 3	33.80±12.22	26.40±3.89	23.90±1.14	28.19±6.18	33.03±2.45	29.98±3.81
Formula 4	69.71±5.49	31.10±2.52	29.48±5.49	25.20±3.73	25.75±0.88	28.81±0.69
Formula 5	42.38±20.01	37.52±22.44	39.04±15.74	25.84±8.77	18.48±0.61	17.61±6.57
Formula 6	39.58±22.34	21.59±1.16	29.40±3.01	33.91±9.60	33.96±7.74	27.60±2.83
Formula 7	32.22±4.90	31.81±10.43	22.36±6.42	23.19±3.97	26.91±0.88	27.53±1.82
Control	0	0	0	0	0	0
MDR *Candida albicans*						
Formula 1	33.45±12.61	27.86±3.33	16.18±0.87	17.43±7.91	16.21±4.02	17.34±0.37
Formula 2	81.39±0.69	71.67±9.29	73.56±11.08	74.78±4.52	73.24±1.83	71.67±3.39
Formula 3	44.87±5.61	41.07±13.64	31.77±9.95	40.78±4.64	35.66±8.49	29.85±2.24
Formula 4	41.73±9.16	20.31±13.81	24.34±6.36	21.85±4.07	12.74±5.96	12.40±3.38
Formula 5	72.13±6.95	43.64±5.54	30.60±6.67	24.28±4.26	15.00±7.07	12.11±6.26
Formula 6	61.47±5.44	46.90±0.40	32.15±4.25	26.55±1.16	15.76±12.49	12.59±3.32
Formula 7	63.90±3.45	34.31±9.03	32.75±8.29	21.74±5.16	23.44±3.55	21.19±9.74
Control	0	0	0	0	0	0

Discussion

Turmeric rhizomes are known for their high content of phenolic compounds, with curcuminoids as the primary components and p-coumaric acid, ferulic acid, and vanillic acid as minor components (Yang et al. 2020). The significantly higher TPC of turmeric compared to aromatic ginger in this study is consistent with a previous report by Muflihah et al. (2021). However, the higher TPC of turmeric compared to red ginger is contrary to the results of a study by Sandrasari et al. (2019). The bioactive content of a given plant is widely varied according to geographical, seasonal, and processing factors. Hence, the different TPC values, along with their relative comparison to other plants, are commonly reported. The TPC of formulations (83.52±0.93 mg GAE/g) in the range of those of the components (20.97±0.78 - 625.35±28.39 mg GAE/g) has also been reported for a five-component polyherbal formulation (Hartanti and Hamad 2023). The DPPH scavenging activity of red ginger is attributable to the phenolic compounds, i.e., flavonoids, gingerols, and shogaol. These compounds are capable of scavenging free radicals, as measured by the DPPH scavenging activity assay (Lukiati et al. 2020; Haroen et al. 2024). Our results confirm previous studies that turmeric was a stronger DPPH scavenging agent than aromatic ginger (Muflihah et al. 2021), and red ginger was stronger than both turmeric and aromatic ginger (Sandrasari et al. 2019; Wahyuni et al. 2021).

The combination of ginger, turmeric, and aromatic ginger oil is expected to exert a synergistic effect on the DPPH scavenging activity. However, our results suggested that the mixture of those crude drugs did not result in synergistic effects. The lower DPPH scavenging activity of the mixture formulation compared to that of red ginger indicated that their action might have antagonistic effects. The interaction effects between components in a mixture, whether antagonistic or synergistic, were mainly defined by the number and ratio of components. The presence of multiple bioactives with different antioxidant mechanisms particularly contributed to the antagonism (Paul and Majumdar 2022). The antagonistic effect on DPPH scavenging activity was also found in other polyherbal formulations. An antagonistic effect was also present in a Malaysian polyherbal formulation containing turmeric, Bentong ginger, black pepper, calamansi, and giant bee honey, and also an Indonesian polyherbal containing king of bitter, bitter vines, Java tea, papaya leaf, and pink and blue ginger (Yap et al. 2023; Hartanti et al. 2025). The antagonistic effect on DPPH scavenging activity was also observed in the combination of cinnamon and mint, as well as that of lemon balm and clove, and cinnamon and clove (Mapeka et al. 2022). The presence of various antioxidant compounds from each component in the polyherbal formulation may react with the radical DPPH at different rates, which diminishes the apparent scavenging effect of the mixture. In addition, quenching, pro-oxidant conversion, or complex formation, also collectively reduce the polyherbal activity compared to that of individual components.

The phenolic compounds are likely responsible for the radical scavenging activity of each crude drug (Formulas 1-3). Such a correlation was reported in a Malaysian study in which individual phenolic compounds of red ginger were strongly correlated with their DPPH scavenging activity. The same result was reported for an Indonesian-originated red and aromatic ginger (Ghasemzadeh et al. 2016; Wahyuni et al. 2021). A significant correlation between these two parameters was also reported in a study on Philippine turmeric (Barbosa and Minguillan 2021). However, a very weak correlation between TPC and DPPH scavenging activity in formulations comprised of two or three components (Formulas 4-7) indicates that the antioxidant activity of the formulations is not solely attributable to the presence of phenolic compounds. Other compounds may contribute to exerting the free radical scavenging activity. However, those other antioxidants may exert different

mechanisms, which diminish the overall antioxidant effects of the formulation, as predicted to be antagonistic by the difference method in the previous section. In addition to phenolic compounds, red ginger and aromatic ginger also contain antioxidant essential oils and terpenoids (Wang et al. 2021; Zhang et al. 2022). Turmeric contains compounds with profound DPPH scavenging activity, including curcuminoids and essential oils, which have different mechanisms due to their distinct structures (Lukitaningsih et al. 2020). These compounds may dynamically interact and eventually modify the antioxidant properties of the polyherbal formulations, which differ from those of monoherbal ones (Heinrich et al. 2023). In general, the correlation between TPC and the antioxidant activity of polyherbal formulations varied widely depending on several factors. The antioxidant potency of phenolic compounds is widely varied, and the TPC assay using the Folin-Ciocalteu reagent does not distinguish between highly active phenolics and those with weaker activity. Moreover, non-phenolic antioxidants also contribute significantly to DPPH scavenging activity but are not reflected in TPC values. Interactions among components may also be synergistic or antagonistic. A Malaysian-originated herbal honey showed a moderate negative correlation between these two parameters. Similar to our results, a weak correlation was observed in a formulation consisting of Java tea, turmeric, and seed-under-leaf. On the other hand, an Indian commercial antidiabetic formulation showed a strong positive correlation between TPC and DPPH scavenging activity (Paul and Majumdar 2023; Yap et al. 2023; Hartanti et al. 2024).

The distillation yield of essential oils varies significantly and is influenced by various factors, including distillation conditions, distillation method, exogenous factors, harvest time, and intrinsic plant material characteristics (Mugao 2024). The yield of red ginger oil in this study (0.80%) was higher than that of the Medan-originated one, which ranged from 0.14% to 0.28% under the same distillation conditions (Nurjanah et al. 2024). The yield of turmeric oil in this study was lower than that of the Brazilian-grown one (Guimarães et al. 2020). The yield of aromatic ginger oil was comparable to that from Thailand (Hearunyakij and Phutdhawong 2022). *Ar*-curcumene, camphene, geranial, and zingiberene, along with geraniol, geranyl acetate, neral, and *trans*-sabinene hydrate, were commonly identified in red ginger oils obtained from various origins in Indonesia (Lukita et al. 2021; Asoka et al. 2022; Badrunanto et al. 2024). The Chinese turmeric oil, obtained by hydrodistillation, primarily contained ar-turmerone, α-turmerone, and germacrone, whereas the Brazilian oil contained turmerones (Guimarães et al. 2020; Xu et al. 2021). As with red ginger and turmeric oils, the components of the aromatic ginger oil also varied widely. The Indonesian commercial one, distilled oil from Karanganyar, Central Java, Indonesia and the one from Bali, Indonesia, mainly contained pentadecane, ethyl p-methoxycinnamate, and ethyl cinnamate. Interestingly, these three compounds were also primarily found in Chinese-originated derivatives (Muderawan et al. 2022; Wang et al. 2023; Muzzazinah et al. 2024). The major compounds of red ginger, turmeric, and aromatic ginger essential oils possess relatively low vapour pressure ranges. They evaporate readily with steam during distillation and are efficiently extracted in the distillate. As a result, they dominate the oil profile regardless of whether the distillation is done singly or in combination. In addition, most volatile sesquiterpenes and aromatic esters are chemically stable under the distillation conditions. Thus, the chemical integrity of the constituents is preserved, and their distillation behaviour remains unchanged (de Sousa et al. 2023).

The antimicrobial activity of the individual essential oils from red ginger, turmeric, and aromatic ginger has been widely evaluated. The antibacterial activity of the oils is highly related to their chemical constituents, which can be attributed to their geranial and turmerone content. Similar to our results, Indonesian red ginger essential oil, primarily composed of sabinene, geranial, and camphene, exhibited moderate inhibitory activity against *S. aureus* with a MIC of 0.0625%. The Malaysian one, rich in camphene, geranial, and geranyl acetate, shows similar potency against the same bacteria (Sivasothy et al. 2011; Nissa et al. 2018). The Ecuadorian study reported that turmeric leaf essential oil, containing major compounds such as β-pinene, α-phellandrene, o-cymene, eucalyptol, and 2-carene, exhibited weak inhibitory effects on the growth of *S. aureus* (Guerrini et al. 2023). Similar to our results, Indian turmeric oil rich in 1,8-cineole, ar-turmerone, α-turmerone, and β-turmerone exhibited strong antibacterial activity, with an MIC of 0.5 mg/mL (Albaqami et al. 2022). Similarly, the antifungal activity of the essential oils and extracts of red ginger, turmeric, and aromatic ginger was also highly correlated to their bioactive constituents. The Indonesian red ginger ethanolic extract from Medan demonstrated weak antifungal activity against HIV patient-isolated *C. albicans.* The Aceh-originated red ginger oil, rich in camphene and geraniol inhibited biofilm formation of *C. albicans* at 0.5% and caused biofilm degradation at 0.125% (Rinanda et al. 2018; Winarni et al. 2023). Unlike the results in this study, Indian and Ecuadorian turmeric essential oils exhibited weak antifungal activity against *C. albicans,* with MICs of 0.63% and 89.75 mg/mL, respectively. The activity of Indian turmeric oil was attributed to zingiberene, clohexene, 4-methyl-3-(1-methylethylidene), and carene (Sharma et al. 2022; Guerrini et al. 2023). The variability in the antibacterial and antifungal activity of a given essential oil obtained from plants grown in different places is primarily attributed to geographical variation in the chemical composition, which is influenced by the climate, soil type, altitude, harvest time, cultivation practices, and genetic variability of the plant populations (de Sousa et al. 2023). The variability in the constituents and bioactivity of Indonesian ginger oil, Chinese turmeric oil, and Indonesian aromatic ginger oil from different origins has been reported (Qiang et al. 2021; Muderawan et al. 2022; Batubara et al. 2023).

Essential oils exert antimicrobial activity by several mechanisms, i.e., disrupting the bacterial membrane, altering the cell membrane's fatty acid composition, increasing membrane permeability, and inhibiting enzyme activity. Essential oil is a mixture of many compounds; therefore,

the effects are the result of the interactions of all constituents. The antimicrobial activity is commonly attributable to their major compounds (Angane et al. 2022). Hence, the variations in the antimicrobial activity of the essential oils from seven different polyherbal formulations could be attributed to differences in their chemical composition. The highest antimicrobial activity demonstrated by Formula 3 oil may be attributed to 3-carene, which previously showed a considerably high effect against *Pseudomonas lundensis*. 3-Carene disrupted cell membrane integrity and ATPase regulation, resulting in the inhibition of cellular respiratory metabolism (Tang et al. 2022). Turmerones may also contribute to the microbial inhibitory activity of Formulas 2-7. α-Turmerone derivatives effectively caused hyphal deformities, increased membrane permeability, and decreased enzyme activity in *Fusarium oxysporum*. On the other hand, 1,8-cineole caused membrane disruption and leakage of proteins, ATP, and DNA in food-borne pathogenic bacteria, such as *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium* (Sobhy et al. 2023; Yu et al. 2025). Camphene, as the major compound of Formula I, was detected at moderate levels in Formulas 4-6, and has been proven to disrupt cell membranes and modify cell permeability of the microorganisms (El-Hachlafi et al. 2023)

The focus of this study was to compare the antimicrobial activity of each essential oil component with that obtained from the combinations. The higher growth inhibitory effect of Formulas 4, 5, and 7 compared to Formulas 1 and 2 likely represents the synergistic antibacterial activity against MDR *S. aureus*. The antagonistic effect may occur against *C. albicans*, with Formulas 2 and 3 exhibiting a higher inhibitory effect compared to Formulas 4-7. However, our current data cannot be used to confirm this. Further interaction effect evaluations using the checkerboard assay or time-kill assay are required (Hulankova 2024). Theoretically, the synergistic antimicrobial effect of essential oils, either in combination with other essential oils or other antimicrobial agents, may enhance efficacy, broaden the spectrum, and aid in combating cases of antibiotic resistance (Bhattacharya et al. 2021). Our findings suggested that combinations of essential oils from red ginger, turmeric, and aromatic ginger, in ratios of 1:1:1 (Formula 4), 2:1:1 (Formula 5), and 1:1:2 (Formula 7), exhibit an increased antibacterial effect against MDR *S. aureus*. The synergistic effects of essential oil combinations derive from the complex interaction of antimicrobial compounds by the individual components. However, the inhibitory activity of those combinations against *C. albicans* was significantly lower than that of the single essential oils. It represented the antagonistic effects on their antifungal activity. The different interaction antibacterial and antifungal effects of the polyherbal oils were likely related to the various structures of their cell walls. Hence, the polyherbal formulation oils evaluated in this study showed increased antibacterial activity, but did not broaden their spectrum of activity. Some examples of essential oil combinations with synergistic effects include lemongrass and geranium oils against *S. aureus* and *Candida* spp., as well as oils of clove, cinnamon, and eucalyptus against *S. aureus*. Additionally, a combination of clove, lemongrass, and aromatic windflower oils is effective against *Candida* spp. (Ngo-Mback et al. 2020; Amarante et al. 2023; de Lima et al. 2024).

Our results suggest that the polyherbal formulation oils showed higher antibacterial and comparable antifungal activities against MDR *S. aureus* and *C. albicans* compared to their individual components. These oils are potentially developed to be used in combination with the antibiotics against which microbes have developed resistance. 1,8-Cineole and geraniol, which were detected in Formulas 4-7 in this study, were capable of blocking the efflux pump developed by microorganisms during antibiotic resistance. The blocked efflux pump enables the cytoplasmic antibiotic concentration to be maintained and works effectively (Ivanov et al. 2021; de Araújo et al. 2025). In addition, essential oils disrupt the formation of biofilm, the aggregate of microorganisms that adhere to each other on a surface and are embedded within a self-produced matrix of Extracellular Polymeric Substances (EPS), by mechanisms that include initial adhesion inhibition, EPS degradation, and quorum-sensing disruption. Zingiberene, a compound found in red ginger essential oils, which was also detected in Formulas 1, 4-7 ones, inhibited EPS production by *Shewanella putrefaciens*. β-Elemene and caryophyllene of aromatic ginger essential oil, which were also detected in Formulas 3-7 oils, showed high affinity of binding and stability of the molecules with the CrtM and SarA proteins of *S. aureus* that are essential for the formation of biofilm (Zhang et al. 2023; Sagar et al. 2024; Sharma et al. 2024).

This study confirmed the identity of the red ginger, turmeric, and aromatic ginger crude drugs using macroscopic, microscopic, and TLC analyses in accordance with the IHP. The difference in the compositional ratio of those crude drugs in polyherbal formulations affected their bioactivity. While antagonistic interactions reduced the DPPH scavenging activity of the formulations, their essential oils exhibited significant antimicrobial activity against MDR *S. aureus* and *C. albicans.* Formulas 4, 5, and 7 oils, which were mixtures of red ginger, turmeric, and aromatic ginger, showed enhanced antibacterial effects compared to single-plant oils, suggesting synergistic interactions. These findings highlight the therapeutic potential of these polyherbal essential oils for combating MDR bacterial infections. Furthermore, their potent antimicrobial activity supports their application in treating *masuk angin*, aligning with the ethnopharmacological use of these plants and addressing the urgent need for novel strategies against antimicrobial resistance.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the National Research and Innovation Agency for this study through the *Riset dan Inovasi untuk Indonesia Maju* (RIIM) Batch 3 program, under contract number 50/IV/KS/05/2023.

REFERENCES

Afendi FM, Heryanto R, Darusman LK, Syahrir NHA, Bakri R, Qomariasih N. 2016. Jamu informatics: A new perspective in jamu research. CICSJ Bull 34 (2): 47-52. DOI: 10.11546/CICSJ.34.47.

Ahmad RS, Hussain MB, Sultan MT, Arshad MS, Waheed M, Shariati MA, Plygun S, Hashempu MH. 2020. Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: A mechanistic review. Evid Based Complement Alternat Med 2020: 7656919. DOI: 10.1155/2020/7656919.

Albaqami JJ, Hamdi H, Narayanankutty A, Visakh NU, Sasidharan A, Kuttithodi AM, Famurewa AC, Pathrose B. 2022. Chemical composition and biological activities of the leaf essential oils of *Curcuma longa, Curcuma aromatica* and *Curcuma angustifolia*. Antibiotics 11 (11): 1547. DOI: 10.3390/antibiotics11111547.

Amarante JF, da Costa MM, da Silva Almeida JRG, de Oliveira HP, 2023. Synergistic interaction of clove, cinnamon, and eucalyptus essential oils impregnated in cellulose acetate electrospun fibers as antibacterial agents against *Staphylococcus aureus*. Braz J Microbiol 54 (3): 1635-1643. DOI: 10.1007/s42770-023-01048-5.

Angane M, Swift S, Huang K, Butts CA, Quek SY. 2022. Essential oils and their major components: An updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. Foods 11 (3): 464. DOI: 10.3390/foods11030464.

Asoka SF, Wahyuni WT, Batubara I, Wahyudi ST. 2022. Red ginger (*Zingiber officinale* var. *rubrum*): Its essential oil content and potential as an anti-*Propionibacterium acnes* by molecular docking. AIP Conf Proc 2563: 050003. DOI: 10.1063/5.0103211.

Badrunanto, Wahyuni WT, Farid M, Batubara I, Yamauchi K. 2024. Antioxidant components of the three different varieties of Indonesian ginger essential oil: In vitro and computational studies. Food Chem Adv 4: 100558. DOI: 10.1016/j.focha.2023.100558.

Barbosa GB, Minguillan JMO. 2021. Antioxidant activity and total phenolic content of fresh and cured rhizomes of *Curcuma longa* and *Etlingera philippinensis*. Intl Food Res J 28 (4): 839-847. DOI: 10.47836/ifrj.28.4.20.

Batubara I, Badrunanto, Wahyuni WT, Farid M. 2023. Combination of extraction and distillation of red ginger rhizome on the composition of active compounds and tyrosinase inhibitory activity. Intl J Adv Sci Eng Inf Technol 13 (2): 431-437. DOI: 10.21462/ijaseit.v13i2.17606.

Bhattacharya R, Rolta R, Dev K, Sourirajan A. 2021. Synergistic potential of essential oils with antibiotics to combat fungal pathogens: Present status and future perspectives. Phytother Res 35 (11): 6089-6100. DOI: 10.1002/ptr.7218.

de Araújo GA, de Morais Oliveira Tintino CD, Pereira RLS et al. 2025. Toxicological assessment of citral and geraniol: Efflux pump inhibition in *Staphylococcus aureus* and invertebrate toxicity. Toxicol Rep 14: 101917. DOI: 10.1016/j.toxrep.2025.101917.

de Lima PMN, Pereira TC, de Carvalho LS, dos Santos LF, Oliveira CER, de Paula Ramos L, Marcucci MC, Hasna AA, de Oliveira LD. 2024. Antimicrobial and synergistic effects of lemongrass and geranium essential oils against *Streptococcus mutans, Staphylococcus aureus,* and *Candida* spp. World J Crit Care Med 13 (3): 92531. DOI: 10.5492/wjccm.v13.i3.92531.

de Sousa DP, Damasceno ROS, Amorati R, Elshabrawy HA, de Castro RD, Bezerra DP, Nunes VRV, Gomes RC, Lima TC. 2023. Essential oils: Chemistry and pharmacological activities. Biomolecules 13 (7): 1144. DOI: 10.3390/biom13071144.

El Hachlafi N, Aanniz T, El Menyiy N, El Baaboua A, El Omari N, Balahbib A, Shariati MA, Zengin G, Fikri-Benbrahim K, Bouyahya A. 2023. In vitro and in vivo biological investigations of camphene and its mechanism insights: A review. Food Rev Intl 39 (4): 1799-1826. DOI: 10.1080/87559129.2021.1936007.

Ghasemzadeh A, Jaafar HZE, Rahmat A. 2016. Variation of the phytochemical constituents and antioxidant activities of *Zingiber officinale* var. *rubrum* Theilade associated with different drying methods and polyphenol oxidase activity. Molecules 21 (6): 780. DOI: 10.3390/molecules21060780.

Guerrini A, Tacchini M, Chiocchio I, Grandini A, Radice M, Maresca I, Paganetto G, Sacchetti G. 2023. A comparative study on chemical compositions and biological activities of four Amazonian Ecuador essential oils: *Curcuma longa* L. (Zingiberaceae), *Cymbopogon citratus* (DC.) Stapf, (Poaceae), *Ocimum campechianum* Mill. (Lamiaceae), and *Zingiber officinale* R. Antibiotics 12 (1): 177. DOI: 10.3390/antibiotics12010177.

Guimarães AF, Vinhas ACA, Gomes AF, Souza LH, Krepsky PB. 2020. Essential oil of *Curcuma longa* L. rhizomes chemical composition, yield variation and stability. Quim Nova 43 (7): 909-913. DOI: 10.21577/0100-4042.20170547.

Hamad A, Djalil AD, Dewi DYS, Hartanti D. 2021. Development of lemon basil essential oil as a natural chicken meat preservative. IOP Conf Ser: Earth Environ Sci 803: 012028. DOI: 10.1088/1755-1315/803/1/012028.

Haroen U, Syafwan S, Kurniawan K, Budiansyah A. 2024. Determination of total phenolics, flavonoids, and testing of antioxidant and antibacterial activities of red ginger (*Zingiber officinale* var. *rubrum*). J Adv Vet Anim Res 11 (1): 114-124. DOI: 10.5455/javar.2024.k755.

Hartanti D, Chatsumpun N, Sa-ngiamsuntorn K, Supharattanasitthi W, Kitphati W, Peungvicha P. 2023. The pharmacognostic standards, antioxidant and antidiabetic activities, and hepatic safety profile of an Indonesian antidiabetic polyherbal formulation. Indones J Pharm 34 (1): 65-78. DOI: 10.22146/ijp.3243.

Hartanti D, Hamad A. 2023. Antioxidant properties and interaction effects of a novel polyherbal formulation. Curr Trends Biotechnol Pharm 17 (4A): 28-33. DOI: 10.5530/ctbp.2023.4s.87.

Hartanti D, Insani AA, Wahono SK, Hamad A. 2024. Extraction method and crude drug-to-solvent ratio effects on the antioxidant properties and physicochemical profile during storage of a polyherbal formulation extract. BIO Web Conf 135: 06002. DOI: 10.1051/bioconf/202413506002.

Hartanti D, Romdhoni MF, Hamad A. 2025. Crude drug standardization and antioxidant properties of an Indonesian antidiabetic polyherbal formulation and plant constituents. Trop J Nat Prod Res 9 (5): 2074-2080. DOI: 10.26538/tjnpr/v9i5.28.

Hearunyakij M, Phutdhawong W. 2022. Optimization, yield and chemical composition of essential oil from *Kaempferia galanga* L. rhizome: comparative study with microwave assisted extraction and hydrodistillation. J Essent Oil Bear Plants 25 (3): 444-455. DOI: 10.1080/0972060X.2022.2108727.

Heinrich M, Barnes J, Prieto-Garcia J, Gibbon S, Williamson EM. 2023. The complex pharmacology of herbal medicines. In: Heinrich M, Barnes J, Prieto-Garcia J, Gibbon S, Williamson EM (eds). Fundamentals of Pharmacognosy and Phytotherapy. Elsevier, Amsterdam, Netherlands.

Hulankova R. 2024. Methods for determination of antimicrobial activity of essential oils in vitro - A review. Plants 13 (19): 2784. DOI: 10.3390/plants13192784.

Indonesian MoH. 2017. Indonesian Herbal Pharmacopeia. Ministry of Health, Republic of Indonesia, Jakarta. [Indonesian]

Ivanov M, Kannan A, Stojković DS, Glamočlija J, Calhelha RC, Ferreira ICFR, Sanglard D, Soković M. 2021. Camphor and eucalyptol - anticandidal spectrum, antivirulence effect, efflux pumps interference and cytotoxicity. Intl J Mol Sci 22: 483. DOI: 10.3390/ijms22020483.

Ju J, Xie Y, Yu H, Guo Y, Cheng Y, Qian H, Yao W. 2022. Synergistic interactions of plant essential oils with antimicrobial agents: A new antimicrobial therapy. Crit Rev Food Sci Nutr 62 (7): 1740-1751. DOI: 10.1080/10408398.2020.1846494.

Kumar A. 2020. Phytochemistry, pharmacological activities and uses of traditional medicinal plant *Kaempferia galanga* L. - an overview. J Ethnopharmacol 253: 112667. DOI: 10.1016/j.jep.2020.112667.

Lukiati B, Sulisetijono, Nugrahaningsih, Masita R. 2020. Determination of total phenol and flavonoid levels and antioxidant activity of methanolic and ethanolic extract of *Zingiber officinale* Rosc var. *rubrum* rhizome. AIP Conf Proc 2231: 040003. DOI: 10.1063/5.0002657.

Lukita S, Khosasi W, Susanto C, Florenly. 2021. The antibacterial effectiveness of red ginger (*Zingiber officinale* Roscoe) essential oil in inhibiting the growth of *Staphylococcus aureus* and *Streptococcus mutans*. Biomed J Indones 7: 364-373. DOI: 10.32539/BJI.v7i2.305.

Lukitaningsih E, Rohman A, Rafi M, Nurrulhidayah AF, Windarsih A. 2020. In vivo antioxidant activities of *Curcuma longa* and *Curcuma xanthorrhiza*: A review. Food Res 4 (1): 13-19. DOI: 10.26656/fr.2017.4(1).172.

Mapeka TM, Sandasi M, Viljoen AM, van Vuuren SF. 2022. Optimization of antioxidant synergy in a polyherbal combination by experimental design. Molecules 27 (13): 4196. DOI: 10.3390/molecules27134196.

Muderawan IW, Mudianta IW, Martiningsih NW. 2022. Physicochemical properties, chemical compositions and antioxidant activities of rhizome oils from two varieties of *Kaempferia galanga*. Indones J Chem 22 (1): 72-85. DOI: 10.22146/ijc.66348.

Muflihah YM, Gollavelli G, Lin Y-C. 2021. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12

Indonesian indigenous herbs. Antioxidants 10 (10): 1530. DOI: 10.3390/antiox10101530.

Mugao L. 2024. Factors influencing yield, chemical composition and efficacy of essential oils. Intl J Multidiscip Res Growth Eval 5 (4): 169-178. DOI: 10.54660/.ijmrge.2024.5.4.169-178.

Murudkar PH, Tambe MS, Chandrasekar SB, Boddeda B, Pawar AT. 2022. Common Ayurvedic, Chinese traditional and Unani antidiabetic formulations - A review. Front Pharmacol 13: 991083. DOI: 10.3389/fphar.2022.991083.

Muzzazinah, Yunus A, Rinanto Y, Suherlan Y, Ramli M, Putri DS, Ningtyas DW, Rahma AL, Nabila SJ. 2024. Profile of chemical compounds and potency of galangal (*Kaempferia galanga* L.) essential oils from Kemuning Village, Karanganyar District, Central Java, Indonesia. Biodiversitas 25 (4): 1386-1393. DOI: 10.13057/biodiv/d250406.

Ngo-Mback MNL, Babii C, Dongmo PMJ, Toghueo MRK, Stefan M, Boyom FF. 2020. Anticandidal and synergistic effect of essential oil fractions from three aromatic plants used in Cameroon. J Mycol Med 30 (2): 100940. DOI: 10.1016/j.mycmed.2020.100940.

Nissa A, Utami R, Sari AM, Nursiwi A. 2018. Combination effect of nisin and red ginger essential oil (*Zingiber officinale* var. *rubrum*) against foodborne pathogens and food spoilage microorganisms. AIP Conf Proc 2014: 020023. DOI: 10.1063/1.5054427.

Nurjanah S, Rosalinda S, Andina DP. 2024. Optimization of red ginger (*Zingiber officinale* var. *rubrum*) extraction using microwave assisted hydrodistillation method. Jurnal Teknik Pertanian Lampung 13 (2): 369-380. DOI: 10.23960/jtep-l.v13i2.369-380.

Orellana-Paucar AM. 2024. Turmeric essential oil constituents as potential drug candidates: A comprehensive overview of their individual bioactivities. Molecules 29: 4210. DOI: 10.3390/molecules29174210.

Paul S, Majumdar M. 2022. Comparative study of six antidiabetic polyherbal formulation for its multimodal approaches in diabetes management. 3 Biotech 12: 114. DOI: 10.1007/s13205-022-03166-7.

Paul S, Majumdar M. 2023. Multimode assessment of commercial polyherbal formulation: An in vitro and in silico approach. Appl Biochem Biotechnol 195 (4): 2261-2281. DOI: 10.1007/s12010-022-04064-7.

Prayoga T, Pradipto YD. 2014. Masuk angin in Javanese healthy lifestyle: A qualitative analysis using health and indigenous psychology approaches. ANIMA Indones Psychol J 29 (3): 146-154. DOI: 10.24123/aipj.v29i3.

Qiang Y, Si R, Tan S, Wei H, Huang B, Wu M, Shi M, Fang L, Fu J, Zeng S. 2021. Spatial variation of volatile organic compounds and antioxidant activity of turmeric (*Curcuma longa* L.) essential oils harvested from four provinces of China. Curr Res Food Sci 4: 882-890. DOI: 10.1016/j.crfs.2021.11.002.

Rahim NFA, Muhammad N, Abdullah N, Talip BA, Poh KH. 2020. The interaction effect and optimal formulation of selected polyherbal extracts towards antioxidant activity. Food Res 4 (6): 2042-2048. DOI: 10.26656/fr.2017.4(6).281.

Rinanda T, Isnanda RP, Zulfitri. 2018. Chemical analysis of red ginger (*Zingiber officinale* Roscoe var *rubrum*) essential oil and its anti-biofilm activity against *Candida albicans*. Nat Prod Commun 13 (12): 1587-1590. DOI: 10.1177/1934578X1801301206.

Sagar PK, Sharma P, Singh R. 2024. Anti-quorum sensing and anti-biofilm activity of ginger (*Zingiber officinale*) rhizomes against multidrug-resistant clinical isolates of *Pseudomonas aeruginosa*. Avicenna J Med Biotechnol 16 (1): 49-56. DOI: 10.18502/ajmb.v16i1.14171.

Sandrasari DA, Sabariman M, Azni IN. 2019. Determination of potential level of Indonesian rhizomes as an antioxidant based on phenolic compound and antioxidant activity. IOP Conf Ser: Earth Environ Sci 383: 012017. DOI: 10.1088/1755-1315/383/1/012017.

Sharma N, Gupta N, Orfali R, Kumar V, Patel CN, Peng J, Perveen S. 2022. Evaluation of the antifungal, antioxidant, and anti-diabetic potential of the essential oil of *Curcuma longa* leaves from the North-Western Himalayas by in vitro and in silico analysis. Molecules 27 (22): 7664. DOI: 10.3390/molecules27227664.

Sharma V, Gogoi B, Borah SN, Ghosh A, Mazumdar A, Kalita RD. 2024. In-silico molecular docking and molecular dynamic simulation of γ-elemene and caryophyllene identified from the essential oil of *Kaempferia galanga* L. against biofilm-forming proteins, CrtM and SarA of *Staphylococcus aureus*. J Biomol Struct Dyn 7: 1-13. DOI: 10.1080/07391102.2024.2310773.

Sivasothy Y, Chong WK, Hamid A, Eldeen IM, Sulaiman SF, Awang K. 2011. Essential oils of *Zingiber officinale* var. *rubrum* Theilade and their antibacterial activities. Food Chem 124 (2): 514-517. DOI: 10.1016/j.foodchem.2010.06.062.

Sobhy M, Ali SS, Cui H, Lin L, El-Sapagh S. 2023. Exploring the potential of 1,8-cineole from cardamom oil against food-borne pathogens: Antibacterial mechanisms and its application in meat preservation. Microb Pathog 184: 106375. DOI: 10.1016/j.micpath.2023.106375.

Tang Z, Chen H, Zhang M, Fan Z, Zhong Q, Chen W, Yun Y-H, Chen W. 2022. Antibacterial mechanism of 3-carene against the meat spoilage bacterium *Pseudomonas lundensis* and its application in pork during refrigerated storage. Foods 11 (1): 92. DOI: 10.3390/foods11010092.

Triratnawati A. 2011. Masuk angin dalam konteks kosmologi Jawa. Humaniora 23: 326-335. DOI: 10.22146/jh.1033. [Indonesian]

Utaminingrum W, Nofrianti, Hartanti D. 2021. Ethnopharmacological study of the polyherbal formula in Baturraden, Indonesia. Suranaree J Sci Technol 28 (5): 070024(1-7).

Wahyuni DR, Widowati W, Kusuma HSW, Pratami HK, Wijayanti CR, Wahyuni CD. 2021. Antioxidant potency of *Kaempferia galanga* Linn and *Zingiber officinale* var. *rubra* rhizomes. 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce), 2021, Medan, Indonesia. DOI: 10.1109/InHeNce52833.2021.9537220.

Wang S-Y, Cai L, Yang N, Xu F-F, Wu Y-S, Liu B. 2023. Chemical composition of the *Kaempferia galanga* L. essential oil and its in vitro and in vivo antioxidant activities. Front Nutr 10: 1080487. DOI: 10.3389/fnut.2023.1080487.

Wang S-Y, Zhao H, Xu H-T, Han X-D, Wu Y-S, Xu F-F, Yang X-B, Göransson U, Liu B. 2021. *Kaempferia galanga* L.: Progresses in phytochemistry, pharmacology, toxicology and ethnomedicinal uses. Front Pharmacol 12: 675350. DOI: 10.3389/fphar.2021.675350.

Widyowati R, Agil M. 2018. Chemical constituents and bioactivities of several Indonesian plants typically used in jamu. Chem Pharm Bull 66 (5): 506-518. DOI: 10.1248/cpb.c17-00983.

Wijayanti II, Budiharjo A, Pangastuti A, Prihapsara F, Artanti AN. 2018. Total phenolic content and antioxidant activity of ginger extract and SNEDDS with eel fish bone oil (*Anguilla* spp.). Nusantara Bioscience 10: 164-169. DOI: 10.13057/nusbiosci/n100306.

Winarni E, Nasution AN, Nasution SW. 2023. Antifungal activity of red ginger (*Zingiber officinale var. rubrum*) and garlic (*Allium sativum*) against HIV patients-isolated *Candida albicans*. Jurnal Penelitian Pendidikan IPA 9 (10): 9038-9044. DOI: 10.29303/jppipa.v9i10.4981.

Xu F-X, Zhang J-Y, Jin J, Li Z-G, She Y-B, Lee M-R. 2021. Microwave-assisted natural deep eutectic solvents pretreatment followed by hydrodistillation coupled with GC-MS for analysis of essential oil from turmeric (*Curcuma longa* L.). J Oleo Sci 70 (10): 1481-1494. DOI: 10.5650/jos.ess20368.

Yang JJ, Rahmawati F. 2022. Antimicrobial effects of various red ginger (*Zingiber officinale*) extract concentrations on *Escherichia coli* bacteria. Eur J Biotechnol Biosci 10: 63-67.

Yang Q-Q, Cheng L-Z, Zhang T, Yaron S, Jiang H-X, Sui Z-Q, Corke H. 2020. Phenolic profiles, antioxidant, and antiproliferative activities of turmeric (*Curcuma longa*). Ind Crops Prod 152: 112561. DOI: 10.1016/j.indcrop.2020.112561.

Yap VL, Tan LF, Rajagopal M, Wiart C, Selvaraja M, Leong MY, Tan PL. 2023. Evaluation of phytochemicals and antioxidant potential of a new polyherbal formulation TC-16: Additive, synergistic or antagonistic? BMC Complement Med Ther 23: 93. DOI: 10.1186/s12906-023-03921-0.

Yu J, Hu W, Zhang X, 2025. Study on the synthesis, structural characterization and antifungal mechanism of ar-turmerone derivatives. J Mol Struct 1346: 142821. DOI: 10.1016/j.molstruc.2025.142821.

Zhang C, Xie Y, Qiu W, Mei J, Xie J. 2023. Antibacterial and antibiofilm efficacy and mechanism of ginger (*Zingiber officinale*) essential oil against *Shewanella putrefaciens*. Plants 12 (8): 1720. DOI: 10.3390/plants12081720.

Zhang S, Kou X, Zhao H, Mak K-K, Balijepalli MK, Pichika MR. 2022. *Zingiber officinale* var. *rubrum*: Red ginger's medicinal uses. Molecules 27 (3): 775. DOI: 10.3390/molecules27030775.