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Altitude-dependent Variation in Antibacterial Properties of Red Ginger (*Zingiber officinale* var. *rubrum*): Implications for Natural Anti-*Salmonella* Agents

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Abstract. Red ginger contains diverse bioactive compounds with strong antioxidant and antibacterial activities. This study investigated the influence of growth location on the anti-*Salmonella* activity of red ginger extracts from seven regions in Java, Indonesia. The fractions were analyzed for total phenolic and flavonoid contents, as well as antioxidant capacity using DPPH and FRAP assays. Antibacterial activity was assessed against *Salmonella* using the Kirby-Bauer disk diffusion method. Results showed that methanol and ethyl acetate fractions exhibited the highest phenolic and flavonoid contents, while the chloroform fraction demonstrated the strongest radical scavenging activity. Extracts from Bumiaji and Lendah displayed the most potent anti-*Salmonella* activity (inhibition zone: 10.08 to 18.00 mm). These findings highlight that altitude and

solvent polarity influence red ginger bioactivity, supporting its potential as a natural antibacterial source.

Keywords: altitude, disk diffusion, solvent fractionation, *Zingiber officinale* var. *rubrum*

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1. Introduction

Salmonellosis remains a major health problem in the developing countries, including Southeast Asia [1], due to foodborne transmission [2][3] and rising antibiotic resistance [4]. In Indonesia and neighboring, *Salmonella* has developed multidrug resistence to first line anti-typhoid, such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol [5], prompting the use of floroquinolones and third-generation cephalosporins [1]. Unfortunately, resistance to these agents has also emerged [6]. This growing resistance underscores the urgent need for alternative antibacterial strategies.

This challenge has driven the search for alternatives antibacterials from medicinal plants [7][8]. Red ginger is a promising candidate [9], as its rhizome is rich in phenolic, flavonoid, terpenoid, etc [10] with broad biological activities, including antibacterial effects [11]. Red ginger ethanolic extract have the inhibition activities against *Staphylococcus aureus*, *E. coli*, *Propionibacterium acnes*, *Streptococcus pyogenes* [10], *Pseudomonas aeruginosa*, and *Bacillus subtilis* [9]. The synergistic use of garlic and ginger oils has been shown to suppress *Salmonella* growth in contaminated poultry meat [12].

A major challenge in utilizing red ginger as an antibacterial agent is the variability of its bioactive composition, which is strongly influenced by growth environment and geographical conditions [13]. Environmental factors associated with altitude affect the biosynthesis of phenolic, flavonoids, essential oils, etc [14], leading to differences in antioxidant and antibacterial properties [15]. However, altitude-dependent effects on red ginger metabolite profiles remain poorly understood, highlighting the need for targeted studies to support optimal cultivation and consistent bioactivity [13][16]. Therefore, a systematic evaluation is required to assess how growth location influences the bioactive composition and anti-*Salmonella* activity of red ginger [17] through Kirby-Bauer method. This study investigates the anti-*Salmonella* activity of methanolic extracts from different locations, to support standardization and potential application of red ginger as a natural antibacterial agent.

2. Methods

2.1. Collection of Red Ginger's Rhizomes

Rhizomes were collected from local farmers between August 2022 and May 2023 at Pakem-Sleman (PS), Pengasih-Kulonprogo (PK), and Lendah-Kulonprogo (LK) (Special Region Yogyakarta); Sumowono-Semarang (SS), Kaloran-Temanggung (KT), and Kajoran-Magelang (KM) (Central Java); and Bumiaji-Batu (East Java), covering altitudes from 17 m (LK) to 1300 m above sea level (BB).

2.2. Rhizomes Extraction and Fractionation

Sliced rhizomes were dried, powdered, and macerated with methanol (1:10, w/v) for 24 h, repeated twice, following a a previously described methods [18]. The combined extracts were concentrated under reduce pressure using rotary evaporator (DLab). The crude extract was then subjected to liquid-liquid partitioning according to a published procedure [19], sequentially fractionated with n-hexane, ethyl acetate, chloroform, methanol, and distilled water to obtain fractions of differing polarity. Each solvent fraction was collected and evaporated under reduced pressure.

2.3. Measurement of Total Phenolic Content (TPC)

The TPC was determined using the Folin-Ciocalteu method [20] with gallic acid as the standart. The extract (1000 µg/mL) was reacted with Folin-Ciocalteu (Merck) and 7% Na_2CO_3 (Merck), incubated at

room temperature for 30 min. Absorbance was recorded at 730 nm with spectrophotometer (Hitachi UH5300). All experiments were performed in triplicate.

2.4. Measurment of Total Flavonoid Content (TFC)

The TFC was analyzed following a published method [20] with quercetin (Merck) as the standart. The extract (1000 µg/mL) was reacted with 10% $AlCl_3$ (Merck) and 1M CH_3COONa (Merck) in methanol, incubated at room temperature for 30 min. Absorbance was measured at 428 nm with spectrophotometer (Hitachi UH5300). All experiments were conducted in triplicate

2.5. Measurement of Antioxidant Activity

DPPH radical scavenging activity was evaluated as previously described [21]. A 0.1 mM DPPH solution in methanol was mixed with extracts (5-25 µg/mL), incubated in the dark at room temperature for 30 min. Absorbance was measured at 516 nm using spectrophotometer (Hitachi UH5300). All experiments were conducted in triplicate. The scavenging activity (%) was calculated as:

$$\%\ inhibition = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control}\ x\ 100$$

The IC_{50} values were determined from the calibration curve equation derived from the inhibition percentages.

Ferric reducing power (FRAP) was determined according to a published method [22]. The extract was reacted with 0.2 M phosphate buffer (pH 6.6) and 1% $K_3Fe(CN)_6$ (Sigma Aldrich), incubation at 50ºC, treated with TCA (Merck), centrifuged, and the supernatant was mixed with 0.1% $FeCl_3$ (Merck). Absorbance was measured at 720 nm using spectrophotometer (Hitachi UH5300). All analysis were performed in triplicate.

2.6. Antibacterial Test

The antibacterial activity was assessed based on previous study [23]. *Salmonella* sp. (FNCC 0050) was cultured on Mueller Hinton medium and paper discs (6 mm) (Macherey-Nagel) were loaded with extract solution (25-100% in 5% DMSO). Chloramphenicol (1 µg/10 µL) and DMSO (5%) served as positive and negative controls, respectively. Plates were incubated at 37ºC for 24 h. The inhibition zones were determined (**Figure 1**) following the previous procedure [24]. Each test was performed in triplicate.

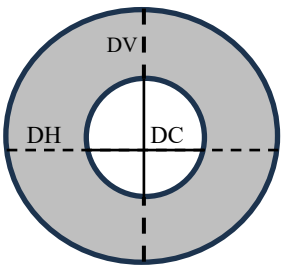

Figure 1. Measurement of the inhibition zone diameter of *Salmonella* sp.

The inhibition zone was calculated using the following formula:

$$Inhibition\ zone\ (mm) = \frac{(\mathrm{DV} - \mathrm{DC}) + (DH - DC)}{2}$$

Where:

- DV : vertical diameter (mm)
- DH : horizontal diameter (mm)
- DC : disc diameter (mm)

2.7. Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a significance level of $p<0.05$. Statistical analysis were performed with SPSS software, version 24 (IBM Corp, Armonk, NY, USA).

3. Results and Discussion

3.1. Phytochemical Activity

To elucidate the bioactive potential of red ginger, the crude methanolic extract was fractionated using solvents of different polarities to assess the distribution of phytochemicals and their functional contributions. Liquid-liquid partitioning enabled correlation between solvent polarity, phytochemical composition, and antioxidant activity [25]. The extraction and fractionation of phytochemicals is strongly influences by their chemical nature, and solvents of different polarities are required since no single solvent can efficiently recover all phytoconstituents and antioxidant compounds from plant materials [26]. In this study, solvents with different polarities were employed the evaluate variations in total phenolic content, total flavonoid content, and antioxidant activity.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH IC_{50} and FRAP) of each fraction of red ginger methanolic extract

Fraction	TPC (µg GAE/mL)	TFC (µg QE/mL)	DPPH IC_{50} (µg/mL)	FRAP (µg/mL)
N-hexane	72.839 ± 0.02^{a}	10.251 ± 0.04^{b}	238.577 ± 1.74^{c}	0.081 ± 0.08^{b}
Ethyl acetate	194.103 ± 0.04^{c}	9.791 ± 0.04^{b}	54.512 ± 1.37^{b}	0.089 ± 0.09^{b}
Chloroform	155.023 ± 0.02^{b}	5.201 ± 0.03^{a}	44.411 ± 0.90^{a}	0.082 ± 0.08^{b}
Methanol	198.356 ± 0.01^{c}	9.393 ± 0.04^{b}	54.540 ± 1.10^{b}	0.084 ± 0.08^{b}
Distilled water	146.287 ± 0.04^{b}	3.460 ± 0.00^{a}	322.619 ± 0.60^{d}	0.058 ± 0.06^{a}

Different superscript letters within the same column indicate significant differences at $p < 0.05$ according to Duncan's Multiple Range Test

Table 1 shows that the methanol fraction contained the highest TPC, although it was not significantly different from the ethyl acetate fraction. Similarly, the highest TFC was observed in the n-hexane fraction, with no significant difference compared to ethyl acetate. In terms of antioxidant activity, the chloroform fraction exhibited the lowest IC_{50} value, indicating the strongest radical scavenging capacity, which was significantly different from the other fractions. Meanwhile, FRAP values indicated relatively low reducing power, with no significant differences observed among all fractions.

These results suggest that solvent polarity plays a critical role in extracting bioactive compounds with different chemical properties [23]. The high phenolic and flavonoid levels in methanol, ethyl acetate, and n-hexane fractions indicate that these solvents are more efficient in extracting polyphenolic and flavonoid constituents of red ginger. The strong radical scavenging activity observed in the chloroform fraction may be attributed to the presence of specific lipophilic compounds that are more soluble in semi-polar solvents. However, the relatively low and non-significant differences in FRAP values among fractions suggest that reducing power may not be the primary antioxidant mechanism in red ginger.

The methanol, effectively extracts phenolic compounds suggests that methanolic extract of red ginger may exhibit stronger antibacterial activity. Phenolic compounds contribute to bacterial growth inhibition through enzyme inactivation, cell wall disruption, and interference of microbial metabolism [27]. Thus, the high phenolic content in methanolic fractionation highlighs red ginger potential as antibacterial agent against pathogenic bacteria such as *Salmonella* sp.

3.2. Antibacterial Activity

The antibacterial activity was strongly influenced by the bioactive compound composition. These are

likely associated with environmental and geographical factors, such as altitude, soil type, temperature, and rainfall, which can modulate the accumulation of secondary metabolites and, consequently, their biological activity [13]. Studies on hazelnut have demonstrated that altitude significantly influences total phenolic content, total flavonoid content, and fatty acid composition [28].

Table 2. Inhibition zone diameter and categories of anti-*Salmonella* activity of methanolic extract of red ginger rhizomes from different locations in Java, Indonesia

Sample Code	Location (Province)	Altitude (m a.s.l)	Concentration (%)	Inhibition Zone (mm)	Activity Category*
BB	Bumiaji-Batu (East Java)	1300	25	18.00 ± 7.00	Strong
			50	5.50 ± 0.50	Moderate
			75	10.33 ± 0.58	Strong
			100	20.00 ± 1.73	Strong
SS	Sumowono-Semarang (Central Java)	955	25	2.67 ± 2.31	Weak
			50	8.00 ± 1.00	Moderate
			75	6.17 ± 0.76	Moderate
			100	4.67 ± 1.15	Weak
KT	Kaloran-Temanggung (Central Java)	715	25	3.00 ± 1.00	Weak
			50	4.83 ± 0.76	Weak
			75	7.50 ± 3.50	Moderate
			100	7.67 ± 2.08	Moderate
PS	Pakem-Sleman (Special Region Yogyakarta)	600	25	6.67 ± 2.32	Moderate
			50	8.50 ± 1.00	Moderate
			75	8.00 ± 0.00	Moderate
			100	6.58 ± 1.46	Moderate
KM	Kajoran-Magelang (Central Java)	578	25	8.42 ± 2.43	Moderate
			50	9.50 ± 1.50	Moderate
			75	8.25 ± 0.75	Moderate
			100	8.00 ± 0.87	Moderate
PK	Pengasih-Kulonprogo (Special Region Yogyakarta)	400	25	9.50 ± 0.87	Moderate
			50	10.33 ± 1.53	Strong
			75	8.83 ± 2.35	Moderate
			100	7.00 ± 1.00	Moderate
LK	Lendah-Kulonprogo (Special Region Yogyakarta)	19	25	8.08 ± 1.18	Moderate
			50	10.08 ± 0.63	Strong
			75	9.00 ± 1.73	Moderate
			100	7.42 ± 1.28	Moderate

*Activity category was classified based on inhibition zone diameter: weak (≦ 5 mm), moderate (5 – 10 mm), strong (10 – 20), and very strong (≧ 20 mm) [29].

In this study, seven red ginger samples were collected from different altitudinal locations, including BB (1300 m), SS (955 m), KT (715 m), PS (600 m), KM (578 m), PK (400 m), and LK (19 m) above sea level. Since the samples were collected from locations with different altitudes, the variation in total phenolic, flavonoid, antioxidant, and antibacterial activity may be influenced by environmental factors such as elevation, temperature, and soil composition [30]. The results indicated that the red ginger extract from BB exhibited the strongest anti-*Salmonella* activity compared to others. The BB extract showed pronounced inhibition at concentrations of 25%, 75%, and 100%. In contrast, most other samples demonstrated only weak to moderate inhibition. Strong antibacterial effects were also observed in PK (50%) and LK (50%) (**Table 2**). These findings suggest that potent anti-*Salmonella* activity in methanolic red ginger extract is associated with samples collected from both extreme high (BB) and extreme low (LK) altitudes.

Higher elevations are often associated with increased environmental stress, such as lower

temperatures and higher UV radiation, which can stimulate the biosynthesis of phenolic and flavonoid compunds with strong antibacterial properties. Previous study reported stronger antibacterial effects and richer phenolic profiles in *Thymbra* species grown at elevated locations [31]. Conversely, extreme lowland conditions (LK) may also induce distinct metabolic adaptations, leading to the accumulation of bioactive compounds that contribute to antibacterial activity. Therefore, both extreme altitudinal conditions could enhance the production of secondary metabolites responsible for the anti-*Salmonella* effects observed in this study.

The antibacterial activity was evaluated using the Kirby-Bauer disk diffusion method, where the diameter of the inhibition zone (clear zone) served as an indicator of antibacterial efficacy. Larger inhibition zone reflected stronger antibacterial activity, while smaller or absent inhibition zones indicated weaker or negligible effects [32]. This Kirby-Bauer method provides a reliable comparative assessment of the antibacterial potential of extracts or fractions by correlating the size of inhibition zones around the disk with the presence and concentration of bioactive compounds in red ginger rhizomes.

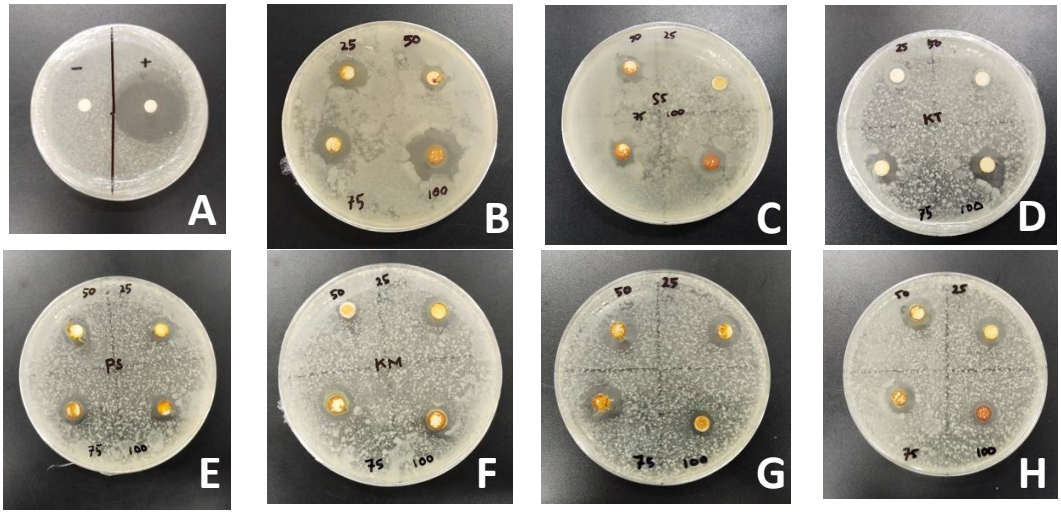

Figure 2. Inhibition zones of methanolic red ginger extracts from seven different locations in Java against *Salmonella* sp. using the Kirby-Bauer disc diffusion method. (A) Positive and negative controls; (B-H) Extracts BB, SS, KT, PS, KM, PK, and LK, respectively

The stronger anti-*Salmonella* sp. activity observed in extracts from extremely high (BB, 1300 m asl) and extremely low (LK, 19 m asl) altitudes may be attributed to variations in their bioactive compound composition, particularly phenolics and flavonoids. Plants growing at high altitudes are often exposed to greater environmental stressors such as lower temperatures, higher UV radiation, and reduced oxygen levels which can stimulate the biosynthesis of secondary metabolites including phenolic compounds with antibacterial properties. On the other hand, plants from lowland areas may experience higher temperature and humidity which can also influence metabolic pathways leading to the accumulation of different bioactive constituents. These variations in phytochemical profiles, contribute to the observed differences in antibacterial activity.

These findings align with earlier studies showing that plants products from higher altitudes rend to accumulate more phenolic and flavonoid compounds, leading to enhanced antioxidant and antimicrobial activities. The altitude influences total phenolic and flavonoid content and also antioxidant activity [33], thus providing support for this study that both high-altitide (BB) and low-altitude (LK) red ginger extracts show strong anti-*Salmonella* sp. activity.

The present study highlights the close correlation between solvent polarity, phytochemical composition, and the biological activities of red ginger extracts. Methanol, as a polar solvent, effectively extracted phenolic compounds, which are known to contribute to strong antibacterial properties.

Similarly, flavonoid-rich fractions, particularly those obtained with semi-polar solvents such as ethyl acetate, demonstrated a notable role in modulating antioxidant potential. The strong radical scavenging and reducing capacities observed in specific fractions suggest that antioxidant activity may synergistically enhance the antibacterial effect, particularly against *Salmonella* sp. These findings indicate that the choice of solvent not only determines the yield and diversity of phenolic and flavonoid compounds but also influences the overall antimicrobial efficacy, underscoring the importance of solvent selection in developing plant-based antibacterial agents.

4. Conclusion

The methanolic extract of red ginger rhizomes showed strong anti-*Salmonella* sp. activity, particularly from samples collected at extreme altitudes. Solvent polarity played a key role in determining phenolic and flavonoid yield, antioxidant potential, and antibacterial effectiveness. It suggests that red ginger is a valuable source of natural antibacterial agents. Further research is needed to isolate active compounds, elucidate their mechanisms, and assess their potential for food preservation and pharmaceutical applications.

Declaration of Competing Interest

The authors declare there is no conflict of interest.

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References

[1] Prabhurajan R, Padmavathy K, Mary R, Selvam E, Govindaraj J. Antibacterial efficacy of the methanols extracts of mango kernel against blood culture isolates of Salmonella Typhi. vol. 14. n.d.

[2] Widyastuti DA. Isolasi DNA Kromosom Salmonella sp . dan Visualisasinya pada Elektroforesis Gel Agarosa 2011:311–7.

[3] Pramiastuti O, S- F, Bhakti Mandala Husada Slawi Stik. Antibacterial activity combined extracts of red ginger (Zingiber officinale var. Rubrum) and betel leaf (Piper betel L.) against Staphylococcus aureus and Escherichia coli. 2020.

[4] Ngemenya MN, Asongana R, Zofou D, Ndip RA, Itoe LO, Babiaka SB. In Vitro Antibacterial Potential against Multidrug-Resistant Salmonella, Cytotoxicity, and Acute Biochemical Effects in Mice of Annona muricata Leaf Extracts. Evidence-Based Complementary and Alternative Medicine 2022;2022. https://doi.org/10.1155/2022/3144684.

[5] Casaux ML, Caffarena RD, Schild CO, Giannitti F, Riet-Correa F, Fraga M. Antibiotic resistance in Salmonella enterica isolated from dairy calves in Uruguay. Brazilian Journal of Microbiology 2019;50:1139–44. https://doi.org/10.1007/s42770-019-00151-w.

[6] Akongwi M, Kwene EC, Awah LA, Tih AE, Ghogomu RT, Cho-Ngwa F, et al. Anti-Salmonella activity on multidrug-resistant strains and cytotoxicity of extracts and constituents of Garcinia brevipedicellata and Garcinia epunctata. Sci Afr 2023;19. https://doi.org/10.1016/j.sciaf.2022.e01465.

[7] Atef M, Ait Chait Y, Ojagh SM, Latifi AM, Esmaeili M, Hammami R, et al. Anti-salmonella activity and peptidomic profiling of peptide fractions produced from sturgeon fish skin collagen (Huso huso) using commercial enzymes. Nutrients 2021;13. https://doi.org/10.3390/nu13082657.

[8] Wang X, Shen Y, Thakur K, Han J, Zhang JG, Hu F, et al. Antibacterial Activity and Mechanism of Ginger Essential Oil against Escherichia coli and Staphylococcus aureus. Molecules 2020;25. https://doi.org/10.3390/molecules25173955.

[9] Zhang S, Kou X, Zhao H, Mak K-K, Balijepalli MK, Pichika MR. Zingiber officinale var. rubrum : Red Ginger's Medicinal Uses. Molecules 2022;27:1–31.
[10] Juariah S, Bakar FIA, Bakar MFA, Endrini S, Kartini S, Ningrum RS. Antibacterial Activity and Inhibition Mechanism of Red Ginger (Zingiber officinale var. rubrum) Ethanol Extract Against Pathogenic Bacteria. Journal of Advanced Research in Applied Sciences and Engineering Technology 2023;30:145–57. https://doi.org/10.37934/araset.30.1.145157.
[11] Kadek N, Sari Y, Agung A, Permatasari AP, Wahyuningsih PA, Geraldi A, et al. Mechanism of Antimicrobial Resistance and Red Ginger as the Solution for Source of Natural Antioxidant: A Brief Review. vol. 34. 2023.
[12] Robinson K, Assumpcao ALF V., Arsi K, Donoghue A, Jesudhasan PRR. Ability of Garlic and Ginger Oil to Reduce Salmonella in Post-Harvest Poultry. Animals 2022;12:2974. https://doi.org/10.3390/ani12212974.
[13] Styawan AA, Purwanto, Susidarti RA, Windarsih A, Rohman A. Comparative Analysis of Essential Oil Profiles From Emprit Ginger Rhizome (Zingiber officinale var. amarum) Grown in Different Locations and Antibacterial Activity Againts Staphylococcus aureus. Indonesian Journal of Tropical and Infectious Disease 2024;12:82–91. https://doi.org/10.20473/ijtid.v12i2.50423.
[14] Haroen U, Syafwan S, Kurniawan K, Budiansyah A. Determination of total phenolics, flavonoids, and testing of antioxidant and antibacterial activities of red ginger (Zingiber officinale var. Rubrum). J Adv Vet Anim Res 2024;11:114–24. https://doi.org/10.5455/JAVAR.2024.K755.
[15] Ghasemzadeh A, Jaafar HZE, Ashkani S, Rahmat A, Juraimi AS, Puteh A, et al. Variation in secondary metabolite production as well as antioxidant and antibacterial activities of Zingiber zerumbet (L.) at different stages of growth. BMC Complement Altern Med 2016;16. https://doi.org/10.1186/s12906-016-1072-6.
[16] Widyastuti DA, Fatimah S, Nuringtyas TR, Santosa D, Rohman A. Total phenolic and flavonoid contents and antioxidant activity of red ginger (Zingiber officinale var. rubrum) rhizomes from seven different regions in the island of Java, Indonesia. Food Res 2025;9:79. https://doi.org/10.26656/fr.2017.9(6).296.
[17] Widyastuti DA, Nuringtyas TR, Santosa D, Rohman A. A perspective on the in vitro tissue culture of red ginger and its impact on antioxidant activity. Food Res 2024;8:193–9. https://doi.org/10.26656/fr.2017.8(4).324.
[18] Widyastuti DA, Fatimah S, Nuringtyas TR, Santosa D, Rohman A. Total phenolic and flavonoid contents and antioxidant activity of red ginger (Zingiber officinale var. rubrum) rhizomes from seven different regions in the island of Java, Indonesia. Food Res 2025;9:79. https://doi.org/10.26656/fr.2017.9(6).296.
[19] Egua MO, Etuk EU, Bello SO, Hassan SW. Antidiabetic potential of liquid-liquid partition fractions of ethanolic seed extract of Corchorus olitorious. Journal of Pharmacognosy and Phytotherapy 2014;6:4–9. https://doi.org/10.5897/JPP2013.0294.
[20] Widyastuti DA, Santosa D, Nuringtyas TR, Rohman A. Callus induction of red ginger (Zingiber officinale var. rubrum) with 2,4-D and Kinetin combination to enhance total phenolic and flavonoid content. IOP Conf. Ser. Earth Environ. Sci., vol. 1364, Institute of Physics; 2024. https://doi.org/10.1088/1755-1315/1364/1/012051.
[21] Nurdyansyah F, Widyastuti DA. Comparison of Antioxidant Activity of Ethanolic, Methanolic, n- Hexan, and Aqueous Extract of Parkia speciosa Peel based on Half -Maximal Inhibitory Concentration Through Free Radical Inhibition. Advance Sustainable Science, Engineering, and Technology 2019;2:0200207–01. https://doi.org/10.30537/sjet.v2i2.
[22] Maryam S, Baits M, Nadia A. PENGUKURAN AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL DAUN KELOR (Moringa oleifera Lam.) MENGGUNAKAN METODE FRAP (Ferric Reducing Antioxidant Power). vol. 2. 2018.

[23] Belhaoues S, Amri S, Bensouilah M. Major phenolic compounds, antioxidant and antibacterial activities of Anthemis praecox Link aerial parts. South African Journal of Botany 2020;131:200–5. https://doi.org/10.1016/j.sajb.2020.02.018.
[24] Magvirah T, Ardhani F, Peternakan Fakultas Pertanian J, Teknologi Hasil Pertanian J. UJI DAYA HAMBAT BAKTERI Staphylococcus aureus MENGGUNAKAN EKSTRAK DAUN TAHONGAI (Kleinhovia hospita L.) Bacterial Inhibitory Test of Staphylococcus aureus Using Leaf Extract of Tahongai (Kleinhovia hospita L.) 2019;2:2019.
[25] Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci 2020;12:1–10. https://doi.org/10.4103/jpbs.JPBS_175_19.
[26] Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Brazilian Journal of Pharmaceutical Sciences 2020;56. https://doi.org/10.1590/s2175-97902019000417129.
[27] Manso T, Lores M, de Miguel T. Antimicrobial Activity of Polyphenols and Natural Polyphenolic Extracts on Clinical Isolates. Antibiotics 2022;11. https://doi.org/10.3390/antibiotics11010046.
[28] Gülsoy E, Kaya ED, Türkhan A, Bulut M, Koyuncu M, Güler E, et al. The Effect of Altitude on Phenolic, Antioxidant and Fatty Acid Compositions of Some Turkish Hazelnut (Coryllus avellana L.) Cultivars. Molecules 2023;28:5067. https://doi.org/10.3390/molecules28135067.
[29] Sakul G, Simbala H, Rundengan G. UJI DAYA HAMBAT EKSTRAK ETANOL DAUN PANGI (Pangium edule Reinw. ex Blume) TERHADAP BAKTERI Staphylococcus aureus, Escherichia coli DAN Pseudomonas aeruginosa THE INHIBITION TEST OF ETHANOL EXTRACT OF PANGI (Pangium edule Reinw. ex Blume) LEAVES AGAINTS Staphylococcus aureus, Escherichia coli AND Pseudomonos aeruginosa. n.d.
[30] Lee J, Jung Y, Shin J, Kim HK, Moon BC, Ryu DH, et al. Secondary Metabolite Profiling of Curcuma Species Grown at Different Locations Using GC/TOF and UPLC/Q-TOF MS 2014:9535–51. https://doi.org/10.3390/molecules19079535.
[31] Khalil N, El-Jalel L, Yousif M, Gonaid M. Altitude impact on the chemical profile and biological activities of Satureja thymbra L. essential oil. BMC Complement Med Ther 2020;20:186. https://doi.org/10.1186/s12906-020-02982-9.
[32] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71–9. https://doi.org/10.1016/j.jpha.2015.11.005.
[33] Suleiman MHA, ALaerjani WMA, Mohammed MEA. Influence of altitudinal variation on the total phenolic and flavonoid content of Acacia and Ziziphus honey. Int J Food Prop 2020;23:2077–86. https://doi.org/10.1080/10942912.2020.1842445.