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Research Article

Drug Alternative Approach Through Comparative Study of Antibacterial Effect of Curcumin and Andrographolide Against *Salmonella enterica* serovar *Typhimurium*

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ABSTRACT

Background: *Salmonella typhimurium* is a pathogen that causes gastroenteritis with a broad host range. Several studies reported antimicrobial resistance against *S. Typhimurium*. The inappropriate use of antibiotics exacerbates the issue of drug resistance. Indonesian natural products, curcumin, a polyphenol derived from turmeric, and andrographolide from Andrographis paniculata, have shown antibacterial activity due to their various health benefits. These natural products are potential candidates for alternative therapy in *S. Typhimurium* infection to evade antibiotic resistance. **Purposes:** This study aims to compare the effectiveness of the antibacterial activity of curcumin and andrographolide against *S. Typhimurium*. **Methods:** This study design was true experimental. The curcumin (500 µg/mL, 1000 µg/mL, 5000 µg/mL) and andrographolide (10 µg/mL, 100 µg/mL, 1000 µg/mL) were tested for their antibacterial effects against *S. Typhimurium* using the Kirby-Bauer diffusion method. Chloramphenicol was used as a positive control, and DMSO was used as a negative control. Inhibition zone bacteria with curcumin treatment compared to andrographolide treatment to assess the effectiveness of the antibacterial activity. **Results:** Various concentrations of curcumin and andrographolide inhibit the growth of *S. Typhimurium* bacteria. The highest average of *S. Typhimurium* inhibition zone was 9 mm and 9.67 mm, with doses of 500 µg/mL curcumin and 1000 µg/mL andrographolide. The andrographolide inhibition zone is larger than curcumin at 1000 µg/mL. These findings showed the potential of andrographolide as a natural antibacterial agent against *S. Typhimurium*. However, chloramphenicol inhibition zone is still highest between andrographolide and curcumin group doses. **Conclusion:** Andrographolide has a more effective antibacterial effect against *S. Typhimurium* than curcumin at 1000 µg/mL based on inhibition zone diameter results. However, chloramphenicol is still more effective as an antibacterial agent against *S. Typhimurium*.

Keywords: andrographolide, antibacterial, curcumin, salmonella sp

INTRODUCTION

Salmonella Typhimurium (*S. Typhimurium*) is a gram-negative pathogenic bacteria found mostly in the intestinal lumen with common symptoms are diarrhea, fever, and stomach pains. Symptoms often appear 6 hours to 6 days after infection and continue to 7 days. This bacteria has wide host range, causes self-limiting gastroenteritis in humans; these are known as "non-typhoidal" serovars (1). The infection mostly via foodborne transmission and remains a major public health, even in developed countries, including Indonesia (2). CDC annually reported several cases contamination of *S. Typhimurium* outbreak investigation linked to food and animal (3). Salmonella's capacity to withstand environmental pressures makes it difficult to remove the disease from the food chain.

In certain circumstances, the infection causes severe diarrhea and needs hospitalized. In occasional cases, systemic infection also appears and needs antibiotics treatment. The appropriate and rational use of antibiotics is the primary way to optimize antibiotic treatment, resulting in a positive impact therapy (4). However, inappropriate use of antibiotics has various problems, such as non-curable diseases, increased risk of drug side effects, possible increase in medical costs, and drug resistance (5). The use of antibiotics is known to trigger the formation of resistance in bacteria as it develops. Antibiotic resistance against *S. Typhimurium* were reported in several studies (6,7). Tetra-resistant and penta-resistant were the most often reported (43%) antibiotic resistance patterns in *S. Typhimurium*. Therefore, alternative drug should be discovered to evade the antibiotic resistance.

Indonesian's natural products have many pharmacological effects that can be used as an alternative or adjunctive therapy. Curcumin is a natural product with many pharmacological effects, including antioxidant, anti-inflammatory, anti-cancer, and antibacterial. Curcumin is a type of polyphenol derived from turmeric rhizome (8,9). Several studies showed the antibacterial effect of Curcumin through in vitro studies (10–14). Adamczak studies results presented curcumin antibacterial effective concentration at 2000 µg/mL and 3000 µg/mL against several Gram-positive bacteria (*Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, Micrococcus tetragenus, M. luteus*) and several Gram-negative bacteria (*E.coli, P. aeruginosa, A. baumannii, Acinetobacter lwoffii*) (12). In addition, Gulel studies showed minimum inhibitor concentration (MIC) of curcumin against *S. Typhimurium* at 362 µg/mL (15). The study by Febriza et al found that curcumin 200 mg/kg BW and 400 mg/kg BW inhibit the growth of S. typhi colony and significantly raised serum vitamin D receptor levels (16). The antibacterial activity of curcumin against S. Typhimurium by inducing the expression of the mRNA CAMP gene, leading to the eradication of the bacteria, was also demonstrated in another study by Febriza et al.(17) Curcumin's anti-inflammatory effect was demonstrated in Simanjuntak et al study that 125mg/kg Curcuma longa extract reduced TNF-alpha and IgG-IgM antibody levels in pregnant mice with acute toxoplasmosis (18,19).

Another natural product that exerts an antibacterial effect is andrographolide, the main bioactive compound in the Sambiloto plant (*Andrographis paniculata*), largely accumulates in leaves, the roots and stem also contain with slighter amounts (20). Irianti studies found that Andrographolide content was 5.72% of Sambiloto leaves ethanolic extracts (21). Several in

vitro studies showed antibacterial activity of andrographolide against *Pseudomonas. aeruginosa, K. pneumoniae E. faecalis, V. cholerae S. pyogenes S. Paratyphi B, Candida albicans, E. coli, Salmonella typhimurium, and Staphylococcus aureus* (20,22,23). Although, both the curcumin and andrographolide have the antibacterial activity, the therapy should use a single bioactive compound. Therefore, this study aims to compare the efficacy of curcumin and andrographolide against *S. typhimurium* and their potential as alternative candidate antibiotic to evade antibiotic-resistance infection through in vitro study. This study also contributes to understanding the antibacterial mechanisms of these natural compounds This data results could be used for further research to explore the potential synergistic effects of combining curcumin and andrographolide to enhance their antibacterial activity against *S. Typhimurium.*

METHODS

Curcumin (C1386-10G) and andrographolide (365645-100MG) were purchased from Sigma-Aldrich and stored at 20°C until the experiment. DMSO solution was purchased from Sigma Aldrich. The chloramphenicol 30 µg as a positive control was purchased from Thermo Fisher Scientific. A series concentration of curcumin (500 µg/ml, 1000 µg/ml, and 5000 µg/ml) and andrographolide (10 µg/ml, 100 µg/ml, and 1000 µg/ml) were dissolved in 10% DMSO solution. This study design was true experimental. The Antibacterial activity of curcumin and andrographolide was assessed against *Salmonella typhimurium* bacteria through an in vitro study, with the DMSO solution used as a negative control.

The *S. Typhimurium* ATCC 14028 in the form of lyophilization were stocked with 15% glycerol and stored at 80°C. Before the study, the bacteria from glycerol stock were sub-cultured on a nutrient agar (NA) plate and incubated for 24 hours at 37°C. After incubation, the presence of *S. Typhimurium* colonies was marked with greyish-white colonies. Gram stain was also carried out to confirm the *S. Typhimurium*. Bacterial suspension was prepared by dissolved bacterial colonies in 0.9% NaCl. The turbidity of the bacterial suspension was compared with the 0.5% McFarland standard solution.

The antibacterial activity test was carried out by the Kirby-Bauer diffusion method. These methods used a paper disc that soaked overnight in curcumin solutions of 500 µg/ml, 1000 µg/ml, and 5000 µg/ml, andrographolide solutions of 10 µg/ml, 100 µg/ml, and 1000 µg/ml, chloramphenicol 30 µg as a positive control, and 10% DMSO as a negative control. Amount bacterial suspension was spread into an MHA plate and incubated for 24 hours at 37°C. After 24 hours, paper discs were placed in the MHA plate containing cultured bacteria and incubated for 24 hours at 37°C. The antibacterial activity was assessed by inhibition zone results of bacteria. The vertical and horizontal diameters were measured as inhibition zones using a ruler. Inhibition zones were calculated using the following formula:

$$\frac{Vertical\ Diameter\ (mm) + \text{Horizontal Diameter (mm)}}{2}$$

Statistical analysis was carried out by SPSS software. Normality data results were analyzed by the Shapiro-Wilk test. Data results considered distributed normally with p-value

> 0.05. Non-parametric tests were conducted using the Kruskal – Wallis test followed by post-hoc analysis using the Pairwise Comparison test. Statistically significant considered with p-value > 0.05.

RESULTS

Gram Staining of *S. Typhimurium*

The *S. Typhimurium* bacteria was sub-cultured into Nutrient Agar medium plate. Figure 1a presented *S. Typhimurium* bacteria marked with grayish-white colonies in NA medium plate. *S. Typhimurium* was Gram-negative bacteria presented by Gram-staining (Figure 1b).

Inhibition Zone of Curcumin Against *S. Typhimurium*

The inhibition zone diameter measured in three repetitions for every concentration of curcumin and andrographolide (Figure 2). The average inhibition zone diameter of curcumin 500 µg/mL was the highest among other doses of curcumin (Table 1). Inhibition zone diameter results did not show curcumin dose-dependent effects. The highest doses of curcumin (5000 µg/ml) did not result in the highest inhibition zone among others. Meanwhile, andrographolide of 1000 µg/mL presented the highest average inhibition zone diameter among other doses of andrographolide (Table 1). In line with the results of curcumin, andrographolide inhibition zone results showed non-linear dose-response relationships. Table 1 presents andrographolide 1000 µg/mL inhibition zone diameter results higher than curcumin 1000 µg/mL. Meanwhile, the positive control (chloramphenicol) inhibition zone result was the highest compared to all groups.

Table 1. Curcumin and Andrographolide Inhibition Zone Diameter Against *S. Typhimurium*

Groups	Inhibition Zone Diameter (mm)			Mean ± SD	p
	1	2	3		
Chloramphenicol 30 µg	54	54	48.5	52.16 ± 3.17	0.071
DMSO 10%	0	0	0	0.00 ± 0.00	
Curcumin 500 µg/mL	11	9.5	6.5	9.00 ± 2.29	
Curcumin 1000 µg/mL	9	7.5	0	5.50 ± 4.82	
Curcumin 5000 µg/mL	8.5	8.5	0	5.66 ± 4.90	
Andrographolide 10 µg/mL	12.5	8.5	0	7.00 ± 6.38	
Andrographolide 100 µg/mL	9.5	10.5	7	9.00 ± 1.80	
Andrographolide 1000 µg/mL	14.5	7.5	7	7.33 ± 7.25	

There was no statistical significance difference of inhibition zone diameter results among groups by the Kruskal-Wallis test (p>0.05) (Table 1). Curcumin and andrographolide in various doses presented no statistical significance difference. In contrast, the positive control group showed statistical significance difference compared to curcumin and andrographolide in various doses.

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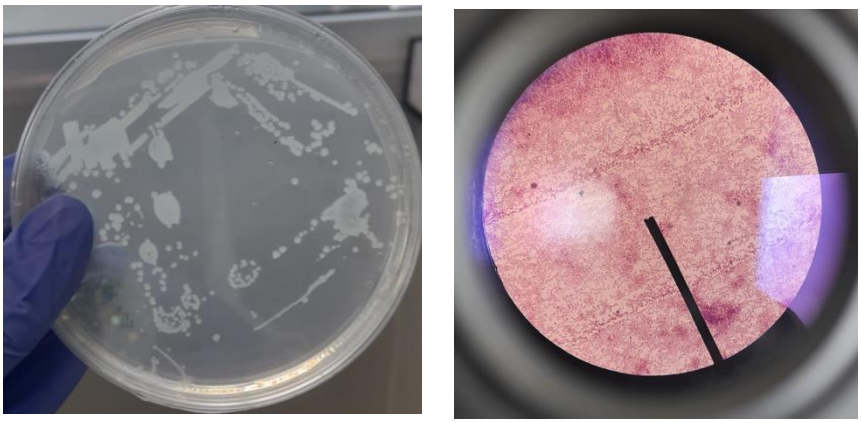

a b

Figure 1. a) Salmonella sp. subculture on nutrient agar b) Gram stain Salmonella sp**.**

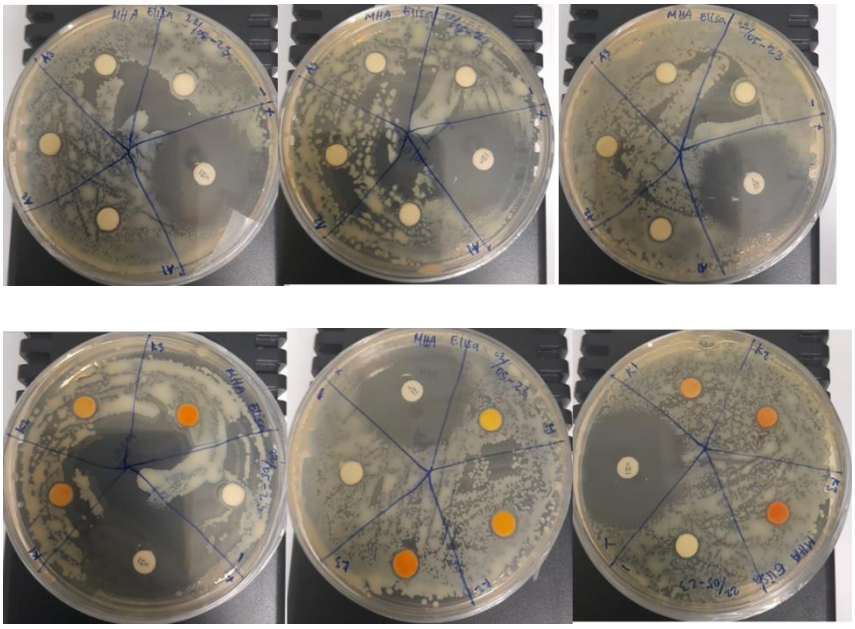

Figure 2. Inhibition zone diameter of curcumin against *S. typhimurium*

DISCUSSION

In this study, the antibacterial activity of curcumin and andrographolide against *S. typhimurium* was assessed by the disk diffusion methods. The inhibition zone diameter of *S. typhimurium* was measured by these methods. From the data obtained, curcumin can inhibit the growth of *S. typhimurium* bacteria and not in a dose-dependent manner. This study proves the results from others studies that used turmeric extract to assess the antibacterial activity. Keyvan study's presents curcumin have antibacterial activity, affecting the growth of *Salmonella enteritidis* bacteria (24). Hussain review study's presents curcumin antibacterial effects against several bacteria, including *S. Typhimurium* through in vitro and in vivo study (25). Setiyawati's studies antibacterial effects of turmeric rhizome extracts showed in dose-dependent manner at

concentrations of 20%, 40%, 60%, and 80% produced inhibition zone *Salmonella sp* bacteria of 6.8 mm, 7.5 mm, 10.3 mm, and 12.3 mm (26). Our results showed 500 µg/mL curcumin against *S. typhimurium* has produced an inhibition zone diameter of 9.00 mm.

Curcumin inhibiting bacterial growth by damaging the cytoplasmic membrane cells resulting disruption of permeability and integrity of cell membranes, leading to bacteria cell death (27,28). In addition, andrographolide also showed inhibition of growth *S. typhimurium* bacteria in dose-dependent manner. Andrographolide compound found in Sambiloto leaf extracts along with flavonoids, tannin, alkaloids, and saponin compound, exerts antibacterial activity to inhibit the growth of *S. typhimurium* (29). Fajar's studies presented that Sambiloto leaf extract at concentrations of 40% and 80% had significant inhibition against *Salmonella sp* bacteria resulting inhibition zone diameter of 9.61 mm and 9.30 mm (30). Meanwhile, this study presented 100 ug/mL Andrographolide inhibition zone diameter of 9 mm. Studies of andrographolide antibacterial activity is still limited. Pharmacological action of andrographolide antibacterial activity through regulating immune response by mechanisms induced Salmonella – specific cell mediated immune response. Several reports of curcumin and andrographolide presented the antibacterial effect against a few positive-gram and negative-gram bacteria. This study presented andrographolide (1000 µg/mL) antibacterial activity against S. typhimurium was more effective than curcumin (1000 µg/mL) based on inhibition zone diameter. This comparative study found that both curcumin and andrographolide have non-linear dose-response relationships. These results can contribute to future research as basic information in dose optimization. The limitations of this study are the need for dose optimization of compounds, the less varied dose range, and the method used. Further studies need to be conducted with broad-range doses of compounds, different methods, and parameters of antibacterial activity to produce comprehensive results.

CONCLUSION

Both Curcumin and andrographolide can inhibit the growth of Salmonella sp based on inhibition zone diameter. Andrographolide has a larger inhibition zone diameter compared to curcumin in the same concentration. Meanwhile, the chloramphenicol still has the highest inhibition zone compare to bioactive compounds in all group doses. These studies concluded that andrographolide has more effective antibacterial activity against S. typhimurium than curcumin but is not competitive with chloramphenicol as antibiotic agent.

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CONFLICT OF INTEREST

This study has no conflict of interest.

REFERENCES

1. Galán JE. Salmonella Typhimurium and inflammation: a pathogen-centric affair. Nat Rev Microbiol. 2022;19(11):716–25.

2. Liu B, Zhang X, Ding X, Bin P, Zhu G. The vertical transmission of Salmonella Enteritidis in a One-Health context. One Heal. 2023;16:100469.
3. Center for Disease Control and Prevention. Reports of Salmonella Outbreak Investigations from 2022. 2022.
4. Bassetti S, Tschudin-Sutter S, Egli A, Osthoff M. Optimizing antibiotic therapies to reduce the risk of bacterial resistance. Eur J Intern Med. 2022;99:7–12.
5. Zarauz JM, Zafrilla P, Ballester P, Cerda B. Study of the Drivers of Inappropriate Use of Antibiotics in Community Pharmacy: Request for Antibiotics Without a Prescription, Degree of Adherence to Treatment and Correct Recycling of Leftover Treatment. Infect Drug Resist ISSN. 2022;15:6773–83.
6. Qin X, Yang M, Cai H, Liu Y, Gorris L, Aslam MZ, et al. Antibiotic Resistance of Salmonella Typhimurium Monophasic and Meta-Analysis. Antibiotics. 2022;11(532): 1–14.
7. Patra SD, Mohakud NK, Panda RK, Sahu BR, Suar M. Prevalence and multidrug resistance in Salmonella enterica Typhimurium : an overview in South East Asia. World J Microbiol Biotechnol. 2021;37(185):1–17.
8. Verma RK, Kumari P, Maurya RK, Kumar V, Verma R, Singh RK. A review on medicinal properties of turmeric (Curcuma longa L.). Int J Pharmacogn Pharm Sci. 2022;4(2):49–53.
9. Puteri FD. Efek Kurkumin Pada Kunyit (Curcuma longa) Sebagai Pengobatan Kanker Lambung. J Ilm Kesehat Sandi Husada. 2020;12(2):860–4.
10. Mody D, Athamneh AIM, Seleem MN. Curcumin: A natural derivative with antibacterial activity against Clostridium difficile. J Glob Antimicrob Resist. 2020 Jun;21:154–61.
11. Hettiarachchi SS, Perera Y, Dunuweera SP, Dunuweera AN, Rajapakse S, Rajapakse RMG. Comparison of Antibacterial Activity of Nanocurcumin with Bulk Curcumin. ACS Omega. 2022;7:46494–500.
12. Adamczak A, Ożarowski M, Karpiński TM. Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals. 2020;13(7):1–12.
13. Akter T, Chakma M, Tanzina AY, Rumi MH, Sharmin M, Shimu S, et al. Curcumin Analogues as a Potential Drug against Antibiotic Resistant Protein , β -Lactamases and L, D-Transpeptidases Involved in Toxin Secretion in Salmonella typhi : A Computational Approach. BioMedInformatics. 2022;2:77–100.
14. Ghasemi M, Khorsandi K, Kianmehr Z. Photodynamic inactivation with curcumin and silver nanoparticles hinders Pseudomonas aeruginosa planktonic and biofilm formation: evaluation of glutathione peroxidase activity and ROS production. World J Microbiol Biotechnol. 2021;37(9):149.
15. Gulel GT, Kanat S, Kucukgoz E. Antibacterial effect of curcumin on Salmonella Typhimurium : In vitro and food model studies. Vet Med (Praha). 2024;2024(4):115–22.
16. Febriza A, Idrus HH, Kasim VN, Natzir R, Hatta M, As S. Curcumin Effects in Inducing Vitamin D Receptor (VDR) and Inhibiting of Salmonella typhi Growth in vivo. Int J Biolife Sci. 2022;1(259):17–24.

17. Febriza A, Natzir R, Hatta M, Uiterwaal CSPM, As S, Alam G, et al. Curcumin effects in inducing mRNA gene cathelidicin antimicrobial peptide in Balb/c mice infected with Salmonella typhi. J Biol Res. 2020;93:76–80.
18. Simanjuntak TP, Hatta M, Rauf S, Prabandari SA, Siagian C. Tumor Necrosis Factor-alpha Levels and Histopathology Finding after Intervention with Curcuma longa Extract. JMedSci. 2018;18(2):56–62.
19. Simanjuntak TP, Hatta M, Tahir AM, Sirait RH, Karo MB, Tambaib T, et al. Analysis of anti-toxoplasma immunoglobulin G and immunoglobulin M antibody levels after intervention with Curcuma Longa extract on early pregnant mice with acute toxoplasmosis. J Glob Infect Dis. 2019;11(1):25–9.
20. Zhang H, Li S, Si Y, Xu H. Andrographolide and its derivatives : Current achievements and future perspectives. Eur J Med Chem. 2021;224(113710):1–21.
21. Irianti IN, Wijayanti AD, Mulyani GT. The Anticoccidial Property of Sambiloto (Andrographis paniculata) Leaf Extract. Indones J Vet Sci. 2022;3(1):8–13.
22. Selvaraj J, Vishnu Priya V, Vijayalakshmi P, Ponnulakshmi R. In silico and in vitro Study on the Inhibition of FtsZ Protein of Staphylococcus aureus by Active Compounds from Andrographis paniculata. J Biol Act Prod from Nat. 2021 Mar;11(2):116–28.
23. Ativui S, Danquah CA, Ofori M, Gibbons S, Bhakta S. Antibacterial and Antifungal Activities of Andrographolide in Combination with Antimicrobial Drugs. 2022;9(July):21–7.
24. Keyvan E, Tutun H, Kahraman HA, Şen E. Determination of time dependent antibacterial activities of curcumin , carvacrol and styrax liquidus on Salmonella Enteritidis. Ankara Univ Vet Fak Derg. 2022;69(4):355–60.
25. Hussain Y, Alam W, Ullah H, Dacrema M, Daglia M, Khan H, et al. Antimicrobial Potential of Curcumin: Therapeutic Potential and Challenges to Clinical Applications. Antibiotics. 2022;11(3).
26. Setiyawati D, Situmeang SMF, Rahmah L, Ningsih SW. Daya hambat ekstrak rimpang kunyit (Curcuma domestica Val .) terhadap pertumbuhan Salmonella typhi. J Prima Med Sains. 2022;4(2):57–60.
27. Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, et al. The Natural Product Curcumin as an Antibacterial Agent : Current Achievements and Problems. Antioxidants 2022,. 2022;11(459):1–21.
28. Teow S, Liew K, Ali SA, Khoo AS, Peh S. Antibacterial Action of Curcumin against Staphylococcus aureus : A Brief Review. J ofTropical Med. 2016;1–10.
29. Zhang L, Zhao H, Bao M, Liu B, Zhang Y, Ji X, et al. Effect of Andrographolide and Its Analogs on Bacterial Infection : A Review. Pharmacology. 2020;100700(576):123–34.
30. Muhammad F. Universitas Muhammadiyah Sumatera Utara; 2018.