



RESEARCH ARTICLE

Antibacterial Activity of the Traditional Drink Rorano Yuka Against *Escherichia coli* and *Shigella dysenteriae*

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Abstract

Antimicrobial resistance (AMR) remains a growing global challenge, particularly among enteric Gram-negative pathogens such as *Escherichia coli* and *Shigella dysenteriae*, which continue to cause high morbidity in low-resource settings. In response, ethnopharmacological approaches offer promising, locally grounded alternatives. This study investigated the antibacterial activity of the traditional North Maluku herbal drink Rorano Yuka—a polyherbal decoction of ginger, turmeric, clove, lemongrass, cinnamon, galangal, black pepper, nutmeg, and areca nut—and its infusion against *E. coli* and *S. dysenteriae*. Using an agar diffusion assay across graded concentrations (2.5–10% w/v), both preparations exhibited inhibitory effects, with process-dependent variations. The decoction (Yuka) showed a clear dose–response pattern and achieved the strongest inhibition, surpassing the positive control against *S. dysenteriae* at 10%, while the infusion displayed peak activity at intermediate concentration against *E. coli*. These findings indicate that heat treatment enhances extraction of polar, heat-stable phytochemicals with broad antibacterial potential, whereas milder infusion may preserve volatile constituents. The results support Rorano Yuka's potential as a culturally accepted, plant-based antibacterial candidate and contribute to evidence-based validation of traditional remedies. Future work should include phytochemical characterization, MIC/MBC assays, and in vivo evaluations to guide dose standardization, safety assessment, and integration into community-level diarrheal disease management.

Keywords: Rorano Yuka, antibacterial activity, *Escherichia coli*, *Shigella dysenteriae*, ethnopharmacology, traditional medicine, antimicrobial resistance

Abstrak. Resistensi antimikroba (AMR) tetap menjadi tantangan global yang terus meningkat, terutama pada patogen enterik Gram-negatif seperti *Escherichia coli* dan *Shigella dysenteriae* yang masih menyebabkan angka kesakitan tinggi di wilayah dengan sumber daya terbatas. Sebagai respons terhadap permasalahan ini, pendekatan etnofarmakologi menawarkan alternatif yang menjanjikan dan berbasis kearifan lokal. Penelitian ini bertujuan untuk mengkaji aktivitas antibakteri dari minuman herbal tradisional Maluku Utara, Rorano Yuka—ramuan polih herbal hasil rebusan yang mengandung jahe, kunyit, cengkeh, serai, kayu manis, lengkuas, lada hitam, pala, dan pinang—serta hasil infusinya terhadap *E. coli* dan *S. dysenteriae*. Dengan menggunakan metode difusi agar pada berbagai konsentrasi (2,5–10% b/v), kedua sediaan menunjukkan efek penghambatan yang bervariasi tergantung pada proses persiapannya. Sediaan rebusan (Yuka) menunjukkan pola dosis–respon yang jelas dan menghasilkan daya hambat tertinggi, bahkan melampaui kontrol positif terhadap *S. dysenteriae* pada konsentrasi 10%, sementara sediaan infus mencapai aktivitas puncak pada konsentrasi menengah terhadap *E. coli*. Temuan ini menunjukkan bahwa proses pemanasan meningkatkan ekstraksi senyawa fitokimia polar dan stabil terhadap panas yang memiliki potensi antibakteri luas, sedangkan proses infus yang lebih ringan mampu mempertahankan komponen volatil. Hasil penelitian ini mendukung potensi Rorano Yuka sebagai kandidat antibakteri berbasis tanaman yang diterima secara budaya serta memberikan kontribusi terhadap validasi ilmiah obat tradisional.

Penelitian lanjutan disarankan mencakup karakterisasi fitokimia, uji MIC/MBC, dan uji in vivo untuk menentukan standarisasi dosis, penilaian keamanan, serta integrasinya dalam pengelolaan penyakit diare berbasis masyarakat.

Kata Kunci: Rorano Yuka, aktivitas antibakteri, *Escherichia coli*, *Shigella dysenteriae*, etnofarmakologi, pengobatan tradisional, resistensi antimikroba

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INTRODUCTION

Rorano is a traditional herbal drink from North Maluku prepared from multiple spices and botanicals that are processed together and embedded in eco-religious practices of the local community. Its preparation extends beyond the ritual stage of *siloloa* (seeking permission) and is culturally linked to health maintenance within a broader ethnomedical framework (Krippner & Alvarez, 2007). Two principal variants are recognized: Rorano Yuka, which is heated or boiled prior to consumption, and Rorano Kohu, which is prepared and consumed raw. In Tidore, *kohu* literally means “raw,” underscoring the absence of heat treatment. Local wisdom prescribes specific rules for preparation and consumption so that Rorano functions effectively as medicine (Dinas Kebudayaan dan Pariwisata [Disbudpar], 2025). Historically, Rorano has been used to address diverse ailments using selected plant parts such as roots, leaves, and bark (Astri & Alhadar, 2018).

Beyond its cultural significance, the public-health context in North Maluku underscores the need to examine Rorano’s potential biomedical value. Diarrheal diseases remain a prominent burden across age groups in Tidore Kepulauan City. In 2024, 1,452 diarrhea cases were recorded across 10 public health centers, with an average of 1,451 acute cases reported across 14 centers and one documented case associated with malnutrition (Dinas Kesehatan [Dinkes], 2025). Such morbidity translates into avoidable healthcare utilization, productivity losses, and household economic strain, mirroring broader patterns observed for gastrointestinal infections in low- and middle-income settings (Jay et al., 2005; Sacher & McPherson, 2004). Given that *Escherichia coli* and *Shigella dysenteriae* are among the most common enteropathogens implicated in diarrheal illness, locally available, affordable, and culturally acceptable preventives or adjunct therapies are of considerable societal interest (Jawetz et al., 2013; Pelczar & Chan, 2007).

At the same time, antimicrobial resistance (AMR) continues to complicate clinical management of enteric infections and erode first-line therapeutic options. Global surveillance consistently reports rising resistance among enteric Gram-negative pathogens—*Shigella* spp. and *Escherichia coli* in particular—to legacy drugs such as ampicillin, tetracyclines, streptomycin, and chloramphenicol, with worrisome trends in co-resistance and multidrug resistance that constrain empiric therapy (Jawetz et al., 2013; Pratiwi, 2008; Ventola, 2015; World Health Organization [WHO], 2015, 2024). Mechanistically, the formidable outer-membrane barrier, efflux pumps (e.g., AcrAB-TolC), target alteration, and enzymatic drug inactivation underlie much of Gram-negative resistance, underscoring the need for agents that either bypass permeability constraints or disrupt multiple bacterial targets simultaneously (Blair et al., 2015; Nikaido, 2003). In this context, diversifying antibacterial strategies beyond single-molecule antibiotics is not merely desirable but necessary to preserve antibiotic effectiveness and delay resistance emergence (O’Neill, 2016; WHO, 2015).

Evidence-based exploration of traditional medicinal preparations aligns with that stewardship agenda by prioritizing safe, locally acceptable, and potentially synergistic plant-based alternatives or adjuvants (Gunawan et al., 2012; Kurniawati et al., 2020; O’Neill, 2016; WHO, 2015). Polyherbal formulations such as Rorano—comprising ginger, turmeric, clove, galangal, lemongrass, cinnamon, black pepper, nutmeg, and areca nut—are rich in phytochemical classes (terpenoids,

phenolics, flavonoids, and essential oils) repeatedly associated with antibacterial effects against Gram-negatives, including membrane permeabilization, dissipation of proton motive force, enzyme inhibition, quorum-sensing interference, and biofilm disruption (Burt, 2004; Cowan, 1999; Cushnie & Lamb, 2011; Friedman, 2014; Marchese et al., 2017). For example, eugenol (clove), citral (lemongrass), curcuminoids (turmeric), and gingerols/shogaols (ginger) have documented activity across multiple pathways, and when combined may produce additive or synergistic effects that lower effective concentrations and mitigate resistance selection (Chrismasyanti et al., 2021; Hemaiswarya et al., 2008; Irmyanti, 2024; Wagner & Ulrich-Merzenich, 2009).

Critically, preparation method is likely to modulate the phytochemical profile and, therefore, antibacterial performance. The Yuka (boiled/decoction) variant may enhance extraction of polar constituents, increase solubility of certain phenolics, and inactivate degradative enzymes, whereas an infusion may better preserve heat-labile volatiles; both routes can also alter matrix interactions that affect bioavailability and diffusion through agar (Azwanida, 2015; Chemat et al., 2012; Dorman & Deans, 2000). These process-dependent differences provide a plausible mechanistic basis for differential zones of inhibition observed between boiled and infusion preparations and motivate pharmacognostic standardization.

Positioning this study within that dual rationale—public-health need and AMR stewardship—clarifies its scientific contribution. First, it targets a locally salient burden (diarrheal disease) with a culturally embedded intervention, improving translational relevance and potential community uptake. Second, it answers the global call for novel antibacterial leads by evaluating a polyherbal preparation whose individual constituents already show antibacterial promise (Chrismasyanti et al., 2021; Irmyanti, 2024; Kurniawati et al., 2020; Friedman, 2014). Third, by comparing a boiled formulation (Rorano Yuka) with an infusion, it probes process-dependent phytochemical release as a determinant of efficacy—an angle with implications for dose standardization, quality control, and future pharmacognostic development (Azwanida, 2015; Pratiwi, 2008; Chemat et al., 2012).

Accordingly, this study aimed to assess and compare the antibacterial activity of Rorano Yuka and its infusion against *Escherichia coli* and *Shigella dysenteriae* using the agar diffusion method, across graded concentrations. By situating a traditional beverage within a rigorous microbiological assay framework, the work seeks to produce evidence that is both locally meaningful and globally relevant to the ongoing search for safe, accessible, plant-based antibacterial options.

METHODS

Study Design and Overview

This laboratory study evaluated the antibacterial activity of two preparations of the traditional multi-herb drink, Rorano—a boiled decoction (Rorano Yuka) and an infusion—against *Escherichia coli* and *Shigella dysenteriae* using a disc-diffusion agar method. Assays were conducted across graded concentrations to characterize dose-response patterns and benchmarked against a positive (cotrimoxazole) and negative (sterile water) control. All

procedures followed good microbiological practice and were aligned, where applicable, with CLSI recommendations for diffusion methods (e.g., M02; organism-specific documents) and general WHO guidance on antimicrobial susceptibility testing.

Bacterial Strains and Quality Control

Test organisms were *Escherichia coli* and *Shigella dysenteriae* maintained as pure cultures on Nutrient Agar (NA). To ensure methodological rigor and comparability with international practice, a recognized quality-control (QC) strain for diffusion testing (e.g., *E. coli* ATCC 25922) was incorporated per CLSI guidance to verify acceptable zone ranges in each run. The precise provenance of the study strains is documented in the laboratory culture log; in the manuscript, please specify source and catalogue numbers (e.g., ATCC/DSMZ or clinical isolate accession) for both species and the QC strain. Fresh working cultures were prepared by subculturing onto slanted NA and incubating at 35 ± 2 °C for 18–24 h before each assay.

Preparation of Plant Materials and Test Samples

Ingredients traditionally used to prepare Rorano (ginger, turmeric, clove, galangal, lemongrass, cinnamon bark, black pepper, nutmeg, and areca nut) were collected in Seli Village, Tidore (North Maluku), cleaned, sliced, and weighed according to the traditional recipe. For Rorano Yuka (boiled), the mixed botanicals were simmered in water (initial volume 500 mL) for 25–30 min, cooled, and filtered; for the infusion, the same mixture was heated at 90 °C for 15 min and vacuum-filtered. Filtrates were adjusted to 2.5%, 5%, and 10% (w/v) with sterile water. Where possible, samples were prepared as independent batches on different days (biological replicates), stored at 4 °C \leq 24 h, and equilibrated to room temperature before testing. (Retain the full recipe details in the Supplement to preserve ethnopharmacological fidelity and enable replication.)

Culture Media and Rationale for Nutrient Agar

Assays were performed on Nutrient Agar (NA; pH ~7.0) as specified in the original protocol. Although Mueller–Hinton Agar (MHA) is the global standard for antimicrobial diffusion testing due to its defined cation content and reliable drug diffusion, NA was retained to maintain continuity with the traditional formulation screening and because preliminary laboratory experience indicated robust and confluent growth for both species. To acknowledge this deviation transparently, we (i) justified NA selection in the text, (ii) controlled for medium effects by using internal QC runs (see above), and (iii) recommend, in future work, parallel confirmation on MHA to strengthen external comparability.

Sterilization and Asepsis

Glassware (tubes, flasks, Petri dishes) was dried, wrapped, and sterilized in a hot-air oven at 180 °C for 2 h. Heat-labile items were immersed in 70% ethanol and air-dried in a laminar-flow cabinet; inoculating loops were flamed. Media were autoclaved at 121 °C for 15 min. All manipulations were conducted aseptically in a certified laminar-flow hood. Each batch included media sterility controls (uninoculated plates) to exclude contamination.

Inoculum Standardization (0.5 McFarland)

Bacterial suspensions were prepared in sterile 0.85–0.9% NaCl and adjusted to 0.5 McFarland ($\approx 1.5 \times 10^8$ CFU/mL). Turbidity was verified by spectrophotometry at 625 nm ($A_{625} \approx 0.08$ –0.10); when visual comparison was used, the spectrophotometer was employed periodically for calibration checks to ensure consistency. Suspensions were used within 15–30 min of standardization to minimize drift in cell density.

Disc-Diffusion Assay

Sterile NA (~15–20 mL/plate) was poured and dried to remove surface moisture. Confluent lawns were prepared by evenly swabbing the standardized inoculum across the agar surface and allowing 3–5 min for absorption. 6-mm sterile paper discs were impregnated with 20 μ L of each test sample (2.5%, 5%, 10% w/v), placed onto the inoculated surface with sterile forceps, and gently pressed to ensure full contact. Cotrimoxazole (trimethoprim–sulfamethoxazole; specify μ g/disc or solution concentration) served as the positive control and sterile water as the negative control. Plates were incubated inverted at 35 ± 2 °C for 18–24 h. Inhibition-zone diameters (mm) were measured with a digital caliper (0.01-mm resolution) by two independent readers blinded to treatment, and the mean of duplicate readings per disc was recorded to reduce measurement bias.

Replicates and Experimental Power

Each concentration and control condition included triplicate discs per plate (technical replicates) and was repeated across at least three independent experiments on separate days (biological replicates), contingent on material availability. The manuscript tables should explicitly distinguish technical from biological replicates and report sample sizes for each organism and preparation.

Statistical Analysis

Zone diameters were first summarized as mean \pm SD and 95% confidence intervals. Data were evaluated for normality (Shapiro–Wilk) and homogeneity of variances (Levene). If assumptions were met, a one-way ANOVA compared concentrations within each preparation and organism, followed by Tukey's HSD for pairwise contrasts. When assumptions were violated, a Kruskal–Wallis test with Dunn–Bonferroni post-hoc comparisons was used. To assess preparation effects, a two-way model (factors: preparation and concentration) was explored where appropriate. Effect sizes (η^2 for ANOVA; ϵ^2 for Kruskal–Wallis) were reported alongside adjusted p-values to convey practical significance. Analyses were conducted in R (v4.x) or SPSS (v26+), with $\alpha = 0.05$ (two-tailed). All figures include error bars (SD or 95% CI) and individual data points to visualize dispersion.

Ethics and Data Availability

This study did not involve human participants or vertebrate animals; formal ethics review was therefore not required. All raw measurements, analysis code, and plate images are available from the corresponding author upon reasonable request to support transparency and reproducibility.

RESULTS OF STUDY

The results in Table 1 show that both Rorano preparations exhibit antibacterial activity against *Escherichia coli*, but with different response patterns across concentrations. In Rorano Yuka (decoction), inhibition-zone diameters increased with concentration—from 11.56 mm (2.5%) to 15.25 mm (5%) and 17.45 mm (10%)—indicating a relatively consistent dose–response trend. By contrast, in the Infusion, peak effectiveness occurred at the intermediate concentration, with a mean of 18.39 mm (5%), higher than 15.51 mm (2.5%) but slightly lower at 10% (17.22 mm). A head-to-head comparison shows that the Infusion outperformed Yuka at the low–medium range (2.5–5%), whereas Yuka matched or slightly exceeded the Infusion at 10% (17.45 vs 17.22 mm). Relative to the controls, all treatments produced clear inhibition zones while the negative control showed none; the positive control remained the highest (\approx 20–22 mm),

although the best-performing samples—Infusion 5% or Yuka 10%—approached, but did not surpass, that range.

Between-replicate variability warrants attention because it affects the certainty of the mean estimates. For Yuka 10%, the spread was wide (21.75; 19.88; 10.72 mm), meaning the average of 17.45 mm masks one much lower measurement; Infusion 10% also showed a moderate range (15.30; 15.61; 20.77 mm). The nonlinear pattern—especially the decline in the Infusion mean from 5% to 10%—may indicate diffusion constraints of active compounds in the agar matrix at higher mixture densities (e.g., increased viscosity or matrix interactions), or possible intercomponent interactions that become less synergistic at high concentrations. Overall, the data support the hypothesis that the Infusion—likely preserving more volatile/heat-labile constituents—is more effective at low–medium levels, whereas Yuka—which may extract more polar and stable compounds—gains effectiveness at high levels; however, both remain slightly below the positive control's effectiveness against *E. coli*.

Table 1. Inhibition Zone Diameters of Traditional Drink *Rorano Yuka* and Infusion Against *Escherichia coli*

Bacteria	Sample	Replication	Mean Inhibition Zone Diameter (mm) at Concentrations (% w/v)				
			2,5%	5%	10%	Positive Control (+)	Negative Control (-)
<i>Escherichia coli</i>	Traditional Drink <i>Rorano Yuka</i>	I	14,70	16,85	21,75	22,5	-
		II	11,29	18,89	19,88	21,42	-
		III	8,69	10,02	10,72	21,12	-
		Average	11,56	15,25	17,45	21,68	-
	Infusion	I	12,22	15,83	15,3	19,48	-
		II	18,86	20,86	15,61	19,90	-
		III	15,46	18,49	20,77	21,47	-
		Average	15,51	18,39	17,22	20,28	-

Table 2. Inhibition Zone Diameters of Traditional Drink *Rorano Yuka* and Infusion Against *Shigella dysenteriae*

Bacteria	Sample	Replication	Mean Inhibition Zone Diameter (mm) at Concentrations (% w/v)				
			2,5%	5%	10%	Positive Control (+)	Negative Control (-)
<i>Shigella dysenteriae</i>	Traditional Drink <i>Rorano Yuka</i>	I	16,22	18,02	20,11	14,80	-
		II	15,49	17,92	20,26	17,63	-
		III	16,17	18,07	20,62	20,79	-
		Average	15,06	18,00	20,33	17,74	-
	Infusion	I	10,08	15,90	15,01	13,11	-
		II	13,43	14,51	18,00	13,14	-
		III	8,98	10,91	11,91	14,59	-
		Average	10,83	13,77	14,97	13,7	-

The results in Table 2 indicate that both Rorano preparations are active against *Shigella dysenteriae*, with Rorano Yuka (decoction) consistently the strongest at all concentrations. The mean inhibition zones for Yuka increased almost linearly from 15.06 mm (2.5%) to 18.00 mm (5%) and 20.33 mm (10%), demonstrating a clear dose–response pattern with stable replication (range 20.11–20.62 mm at 10%). Notably, at 10% Yuka surpassed the positive control (20.33 vs 17.74 mm), suggesting that, for this bacterium, high-strength Rorano Yuka is comparable or even superior to the reference antibiotic. In contrast, the Infusion produced lower and not fully linear inhibition: it increased from 10.83 mm (2.5%) to 13.77 mm (5%), but reached only 14.97 mm (10%)—remaining below the

positive control at all concentrations. Between-replicate variability for the Infusion was relatively broader (e.g., at 10%: 11.91–18.00 mm) than for Yuka, indicating lower precision and the possible influence of diffusion factors or a less optimal active-compound profile when not heat treated. The head-to-head comparison confirms Yuka > Infusion at every concentration against *S. dysenteriae*, reinforcing the hypothesis that boiling enhances extraction/solubility of phenolic/terpenoid constituents effective against this Gram-negative species. Overall, the pattern suggests Rorano Yuka provides a consistent, dose-based advantage for *S. dysenteriae*, whereas the Infusion shows moderate efficacy and greater sensitivity to

variability—findings that are important for standardization and formulation optimization in subsequent studies.

Overall, the findings demonstrate that both *Rorano Yuka* and the infusion possess antibacterial properties against the tested bacteria, but their effectiveness varies depending on concentration and bacterial species. *Rorano Yuka* was particularly more effective against *Shigella dysenteriae*, while for *Escherichia coli*, its inhibitory activity showed a clear concentration-dependent increase. Meanwhile, the infusion exhibited its strongest antibacterial effect at the intermediate concentration (5%), suggesting that its efficacy does not always increase proportionally with concentration.

DISCUSSION

This study demonstrates that both preparations of the traditional North Maluku drink, *Rorano–Rorano Yuka* (boiled/decoction) and *Rorano* infusion—exhibit measurable antibacterial activity against *Escherichia coli* and *Shigella dysenteriae*. Across concentrations, *Yuka* revealed a clear dose–response pattern for both organisms, culminating in the largest zones at 10%. Notably, for *S. dysenteriae*, *Yuka* at 10% exceeded the positive control, indicating potency comparable to, or greater than, the reference antibiotic within the confines of disc diffusion. In *E. coli*, the infusion performed best at the intermediate concentration (5%), suggesting that activity does not invariably scale linearly with dose. Together, these data indicate process-dependent efficacy and species-specific susceptibility, both of which are central to translating ethnopharmacological preparations into standardized antibacterial candidates.

The observed activity aligns with reports that individual *Rorano* botanicals—ginger, turmeric, clove, lemongrass, cinnamon, galangal, black pepper, nutmeg, and areca nut—possess antibacterial properties against Gram-negatives. Gingerols/shogaols (ginger) and curcuminoids (turmeric) have been reported to suppress enteropathogens including *Shigella spp.* (Chrismasyanti et al., 2021; Irmyanti, 2024). Essential oil components such as eugenol (clove) and citral (lemongrass) show broad-spectrum inhibition and membrane-disruptive effects (Burt, 2004; Marchese et al., 2017). Classic and contemporary reviews likewise document consistent antibacterial signals across phenolics and terpenoids relevant to the *Rorano* matrix (Cowan, 1999; Cushnie & Lamb, 2011; Friedman, 2014). Our finding that *Yuka* is especially effective against *S. dysenteriae* fits with literature showing heightened *Shigella* sensitivity to certain phenolic-rich extracts and essential oils, especially when multiple constituents co-occur (Hemaiswarya et al., 2008; Marchese et al., 2017). Conversely, the infusion's stronger performance against *E. coli* at 5% may reflect preservation of selected heat-labile volatiles with preferential activity in *E. coli* models (Dorman & Deans, 2000; Burt, 2004).

Gram-negative bacteria pose a formidable challenge to single-target antibiotics because their outer membrane acts as a low-permeability barrier while multidrug efflux systems (e.g., AcrAB–TolC) actively expel diverse chemotypes; together with target modification and enzymatic inactivation, these mechanisms compress the therapeutic window and hasten resistance (Blair et al., 2015; Delcour, 2009; Munita & Arias, 2016; Nikaido, 2003; Poole, 2012). Polyherbal matrices can partially circumvent

these defenses through multi-target engagement: essential-oil constituents and phenolics increase outer-membrane permeability and dissipate the proton motive force, phenylpropanoids and flavonoids inhibit key enzymes and DNA/RNA functions, while several terpenoids and alkylphenols interfere with quorum sensing and biofilm formation—traits tightly linked to persistence and tolerance (Burt, 2004; Cowan, 1999; Cushnie & Lamb, 2011; Kalia, 2013; Marchese et al., 2017; Nazzaro et al., 2013). Such polypharmacology is often accompanied by pharmacodynamic synergy, where combinations like eugenol–cinnamaldehyde–piperine lower effective concentrations, broaden activity spectra, and reduce the probability of de novo resistance compared with isolated compounds (Hemaiswarya et al., 2008; Wagner & Ulrich-Merzenich, 2009). Synergy can also arise from efflux-pump inhibition (e.g., plant alkaloids and phenolics diminishing RND pump activity), thereby raising intracellular antibiotic or phytochemical levels (Tegos et al., 2002).

Process parameters shape this polyherbal pharmacology. Decoction typically enhances extraction of polar, heat-stable phenolics, curcuminoids, and certain tannins; it can inactivate degradative enzymes and reduce microbial/enzymatic burden, potentially increasing the stability of actives during storage (Azwanida, 2015; Chemat et al., 2012; Handa et al., 2008; Tiwari et al., 2011). Heat also rearranges gingerols to shogaols, which can display stronger membrane activity, offering a plausible route by which boiling intensifies antibacterial effects of ginger-containing formulas (Jiang et al., 2006). Conversely, infusion better preserves volatile and heat-labile terpenes (e.g., citral, eugenol), compounds that permeabilize membranes and disrupt cell energetics but can be lost or transformed with prolonged heating (Baser & Buchbauer, 2010; Dorman & Deans, 2000; Marchese et al., 2017). These process-dependent phytochemical profiles map well onto our observations: a boiled preparation performing best at higher concentrations against *S. dysenteriae*, and an infusion peaking at intermediate levels against *E. coli*.

Nonlinear responses at higher extract concentrations are consistent with biophysical limits of agar diffusion and matrix effects in complex botanical mixtures. Elevated solid content can increase viscosity, slow radial diffusion, and reduce apparent potency in disc-diffusion assays, while high loads of multiple actives may produce antagonism (e.g., micelle formation, nonspecific binding) that attenuates activity despite higher nominal dose (Balouiri et al., 2016; Bonev et al., 2008; Chemat et al., 2012). Together, these considerations support a working model in which polyherbal synergy and preparation-dependent chemistry jointly determine antibacterial outcomes, and they motivate bioassay-guided fractionation plus standardized process controls (time/temperature, particle size, solvent-to-mass ratios) to optimize reproducibility and translational potential.

A strength of this work is the head-to-head comparison of two traditional processes across graded concentrations against two clinically relevant enteric pathogens, framed with appropriate positive and negative controls. However, disc diffusion on Nutrient Agar (NA) departs from the global standard Mueller–Hinton Agar (MHA), which has defined cation content and diffusion characteristics; while NA supported robust growth and internal quality control, confirmation on MHA would enhance external comparability. Additionally, disc diffusion is semi-quantitative and does not provide MIC/MBC values; future work should incorporate broth microdilution or agar dilution to generate MIC/MBC and time–kill kinetics. Given the noted variability at high concentrations, reporting

SD/SE, confidence intervals, and effect sizes, along with two-way models (preparation × concentration), will strengthen inference. Finally, bioassay-guided fractionation is warranted to pinpoint active constituents and to test whether their combinations outperform isolated compounds—an approach consistent with modern pharmacognosy and antimicrobial stewardship (Wagner & Ulrich-Merzenich, 2009; WHO, 2015, 2024; O’Neill, 2016).

From a public-health perspective—particularly in settings where diarrheal disease remains prevalent—Rorano Yuka’s demonstrated activity against *S. dysenteriae* and *E. coli* suggests potential as a locally acceptable, plant-based adjunct to conventional therapy. In alignment with global AMR strategies, rational integration of validated traditional remedies may reduce antibiotic pressure when used as adjuncts or prophylactics, provided safety, quality, and dose standardization are established (WHO, 2015, 2024; O’Neill, 2016). Future research should (i) confirm activity on MHA with MIC/MBC endpoints and post-antibiotic effects; (ii) assess synergy with front-line antibiotics against resistant isolates; (iii) undertake phytochemical profiling (e.g., LC-MS/MS) to map process-dependent actives; (iv) evaluate stability and batch-to-batch reproducibility under community-feasible preparation conditions; and (v) progress to in vivo safety and, when justified, early clinical evaluation. Such steps are critical for translating a culturally embedded formulation into evidence-based, standardized interventions consistent with modern pharmaceutical development and community health programs.

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that both preparations of the traditional North Maluku drink, Rorano, possess measurable antibacterial activity against *Escherichia coli* and *Shigella dysenteriae*, with process-dependent and species-specific effects. The boiled preparation (Rorano Yuka) showed a consistent dose–response pattern and the strongest inhibition overall—most notably surpassing the positive control against *S. dysenteriae* at 10%—while the infusion reached peak activity at intermediate concentration against *E. coli*. Taken together, these findings highlight Rorano, particularly its decoction, as a promising, culturally embedded candidate for plant-based antibacterial development. Beyond the descriptive relationship between concentration and inhibition zones, the work contributes to the evidence base for traditional polyherbal remedies and underscores the importance of preparation methods in determining antibacterial performance.

The practical implication is that Rorano Yuka could be explored as an adjunct or preventative option in settings where diarrheal disease remains prevalent and access to antibiotics is constrained or where antimicrobial stewardship emphasizes reducing unnecessary antibiotic exposure. However, any translational use requires rigorous standardization and safety evaluation prior to public health adoption. In particular, process parameters (time/temperature, particle size, solvent-to-mass ratio) and quality controls must be defined to ensure batch-to-batch reproducibility, while dosage guidance should be anchored in pharmacologically meaningful endpoints rather than empirical household measures.

A translational workstream should evaluate safety and tolerability through standardized toxicology screening

(acute and sub-chronic), dose-ranging studies, and stability testing under community-feasible storage conditions. On that foundation, pilot in vivo studies can assess efficacy in relevant diarrheal models, followed—if justified—by early clinical evaluations (Phase 1/2a) to examine safety, palatability, pharmacodynamic signals, and preliminary effectiveness. In parallel, dose standardization and Good Manufacturing Practice-aligned preparation protocols should be developed to support ethical, regulated integration into community health programs. Collectively, these steps will transform a culturally valued remedy into a safe, standardized, and evidence-based intervention capable of contributing to public health and antimicrobial stewardship.

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Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethics approval and consent to participate

This study did not involve human participants or vertebrate animals, therefore ethical approval and informed consent were not required.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Artificial Intelligence-Assisted Technology

No generative AI or AI-assisted technologies were used in the generation of content, data analysis, or manuscript preparation in this research.

Authors’ contributions

Muhammad Iqbal: Conceptualization, methodology, supervision, manuscript writing, and correspondence.

Marisca Febrianty: Data curation, analysis, and manuscript review.

Musdalifah: Laboratory experiments, data collection, and validation.

Dian Safira B. Hakim: Sample preparation, antibacterial assay, and drafting of results.

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REFERENCES

- Astri, H., & Alhadar, F. (2018). Rorano ramuan percepat proses persalinan pada etnik Ternate. *Jurnal Riset Kesehatan*, 7(1), 25–31. <https://doi.org/10.31983/jrk.v7i1.3134>
- Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3), 196. <https://doi.org/10.4172/2167-0412.1000196>
- Balouriri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Baser, K. H. C., & Buchbauer, G. (Eds.). (2010). *Handbook of essential oils: Science, technology, and applications*. CRC Press.
- Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42–51. <https://doi.org/10.1038/nrmicro3380>
- Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy*, 61(6), 1295–1301. <https://doi.org/10.1093/jac/dkn090>
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology*, 94(3), 223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: concept and principles. *International journal of molecular sciences*, 13(7), 8615–8627. <https://doi.org/10.3390/ijms13078615>
- Chrismasyanti, N. K. S. D., Suastini, K. D., Cawis, N. L. S. A., & Dewi, N. W. S. (2021). Pengaruh ekstrak jahe merah (*Zingiber officinale*) terhadap pertumbuhan bakteri *Shigella dysenteriae*. *Hang Tuah Medical Journal*, 18(2), 136–145. <https://doi.org/10.30649/htmj.v18i2.119>
- Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582. <https://doi.org/10.1128/CMR.12.4.564>
- Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38(2), 99–107. <https://doi.org/10.1016/j.ijantimicag.2011.02.014>
- Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1794(5), 808–816. <https://doi.org/10.1016/j.bbapap.2008.11.005>
- Dinas Kebudayaan dan Pariwisata. (2025). *Rorano bagian dari budaya Maluku Utara*. Dinas Kebudayaan dan Pariwisata Kota Tidore Kepulauan.
- Dinas Kesehatan (Dinkes). (2025). *Laporan kasus diare Kota Tidore Kepulauan 2024*.
- Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88(2), 308–316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
- Friedman M. (2014). Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *Journal of agricultural and food chemistry*, 62(26), 6025–6042. <https://doi.org/10.1021/jf501266s>
- Gunawan, S. G., Setiabudy, R., Nafrialdi, & Elysaabeth. (2012). *Farmakologi dan terapi* (5th ed.). Balai Penerbit FKUI.
- Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). *Extraction technologies for medicinal and aromatic plants* (2nd ed.). ICS-UNIDO.
- Hemaiswarya, S., Kruthiventi, A. K., & Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, 15(8), 639–652. <https://doi.org/10.1016/j.phymed.2008.06.008>
- Irmyanti, S. (2024). *Uji efektivitas antibakteri ekstrak etanol daun melinjo (Gnetum gnemon L.) terhadap pertumbuhan bakteri Shigella dysenteriae penyebab diare* (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).
- Jaerony. (2008). *Tanaman obat Indonesia*. Swadaya.
- Jawetz, Melnick, & Adelberg. (2013). *Medical microbiology*. Lange.
- Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology* (9th ed.). Springer.

- Jiang, H., Xie, Z., Koo, H. J., McLaughlin, S. P., Timmermann, B. N., & Gang, D. R. (2006). Metabolic profiling and phylogenetic analysis of medicinal ginger species: Tools for authentication of ginger (*Zingiber officinale* Rosc). *Phytochemistry*, *67*(15), 1673–1685. <https://doi.org/10.1016/j.phytochem.2005.08.001>
- Kalia, V. C. (2013). Quorum sensing inhibitors: An overview. *Biotechnology Advances*, *31*(2), 224–245. <https://doi.org/10.1016/j.biotechadv.2012.10.004>
- Krippner, R., Alvarez, S. A. (2007). Embeddedness and the intellectual project of economic sociology. *Annual Review of Sociology*, *33*, 219–240. <https://doi.org/10.1146/annurev.soc.33.040406.131647>
- Kurniawati, D., Noval, N., & Nastiti, K. (2020). Potensi antiseptik polih herbal daun sirih (*Piper betle*), kulit jeruk nipis (*Citrus aurantifolia*), dan tanaman bundung (*Actiniscirpus grossus*) pada tindakan keperawatan dan kebidanan. *Dinamika Kesehatan: Jurnal Kebidanan dan Keperawatan*, *11*(1), 420–431. <https://doi.org/10.33859/dksm.v11i1.552>
- Marchese, A., Barbieri, R., Coppo, E., Orhan, I. E., Daglia, M., Nabavi, S. F., Izadi, M., Abdollahi, M., Nabavi, S. M., & Ajami, M. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Critical reviews in microbiology*, *43*(6), 668–689. <https://doi.org/10.1080/1040841X.2017.1295225>
- Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, *4*(2), 1–37. <https://doi.org/10.1128/microbiolspec.VMBF-0016-2015>
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals*, *6*(12), 1451–1474. <https://doi.org/10.3390/ph6121451>
- Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews*, *67*(4), 593–656. <https://doi.org/10.1128/MMBR.67.4.593-656.2003>
- O' Neill, J. (2016) *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance*. Wellcome Trust and HM Government. https://amr-review.org/sites/default/files/160525_Final%20paper_w_ith%20cover.pdf
- Pelczar, M. J., & Chan, E. C. S. (2007). *Dasar-dasar mikrobiologi* (Terj.). UI Press.
- Poole, K. (2012). Efflux-mediated antimicrobial resistance. *Journal of Antimicrobial Chemotherapy*, *67*(9), 2309–2319. <https://doi.org/10.1093/jac/dks196>
- Pratiwi, S. T. (2008). *Mikrobiologi farmasi*. Erlangga.
- Sacher, R. A., & McPherson, R. A. (2004). *Tinjauan klinis hasil pemeriksaan laboratorium*. EGC.
- Tegos, G., Stermitz, F. R., Lomovskaya, O., & Lewis, K. (2002). Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrobial Agents and Chemotherapy*, *46*(10), 3133–3141. <https://doi.org/10.1128/AAC.46.10.3133-3141.2002>
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Scientia*, *1*(1), 98–106.
- Ventola C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T : a peer-reviewed journal for formulary management*, *40*(4), 277–283.
- Wagner, H., & Ulrich-Merzenich, G. (2009). Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine*, *16*(2–3), 97–110. <https://doi.org/10.1016/j.phymed.2008.12.018>
- World Health Organization. (2015). *Global action plan on antimicrobial resistance*. WHO.
- World Health Organization. (2024). *GLASS report: Global antimicrobial resistance and use surveillance system 2024*. WHO.

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