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HARNESSING THE SYNERGY OF NANOEMULGEL OF *CURCUMA XANTHORRHIZA* AND *CURCUMA ZEDOARIA* FOR ENHANCED ANTI-MRSA

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most concerning resistant bacteria, often causing infections that are increasingly difficult to treat. The rapid rise of antibiotic resistance has pushed researchers to seek new strategies, including exploring herbal medicines as alternative or complementary therapies. Among Indonesian medicinal plants, *Curcuma xanthorrhiza* and *Curcuma zedoaria* have long been recognized for their therapeutic value. Both are rich in bioactive compounds such as xanthorrhizol, curcuminoids, and essential oils, which have shown promising antibacterial activity. Yet, their clinical application is limited by poor solubility and low bioavailability, reducing their effectiveness when applied directly.

Nanoemulgel systems provide a way to overcome these barriers. By combining the benefits of nanoemulsions with the convenience of gels, they represent a smart delivery platform for herbal extracts. This study aimed to harness the synergy of *Curcuma xanthorrhiza* and *Curcuma zedoaria* in a nanoemulgel system and evaluate its potential against MRSA. Extracts were prepared by ethanol maceration, incorporated into a nanoemulsion through ultrasonication, and then formulated into a carbopol-based gel. The nanoemulgel was assessed for physicochemical properties and tested against MRSA using microdillution, MIC, and MBC assays.

The results demonstrate that single extracts of *Curcuma zedoaria* and *Curcuma xanthorrhiza* exhibit stronger antibacterial activity against MRSA compared to their combined formulations. The nanoemulgel also demonstrated stable physicochemical characteristics suitable for topical application.

In summary, this dual Curcuma nanoemulgel shows encouraging potential as a natural-based therapeutic approach to combat MRSA infections, bridging traditional herbal wisdom with modern nanotechnology.

Keywords: Nanoemulgel, *Curcuma xanthorrhiza, Curcuma zedoaria,* MRSA, antibacterial synergy

Abstrak

Methicillin-resistant Staphylococcus aureus (MRSA) merupakan salah satu bakteri resisten yang paling mengkhawatirkan, sering kali menyebabkan infeksi yang semakin sulit diobati. Meningkatnya resistensi antibiotik secara pesat mendorong para peneliti untuk mencari strategi baru, termasuk mengeksplorasi obat herbal sebagai terapi alternatif atau komplementer. Di antara tanaman obat Indonesia, Curcuma xanthorrhiza dan Curcuma zedoaria telah lama dikenal karena nilai terapeutiknya. Keduanya kaya akan senyawa bioaktif seperti xanthorrhizol, kurkuminoid, dan minyak atsiri, yang menunjukkan aktivitas antibakteri menjanjikan. Namun, penerapan klinisnya terkendala oleh kelarutan yang buruk dan bioavailabilitas yang rendah, sehingga mengurangi efektivitasnya saat diaplikasikan secara langsung.

Sistem nanoemulgel menawarkan solusi untuk mengatasi hambatan tersebut. Dengan menggabungkan manfaat nanoemulsi dan kenyamanan sediaan gel, sistem ini menjadi platform penghantaran yang cerdas untuk ekstrak herbal. Penelitian ini bertujuan untuk memanfaatkan sinergi antara Curcuma xanthorrhiza dan Curcuma zedoaria dalam sistem nanoemulgel serta mengevaluasi potensinya terhadap MRSA. Ekstrak disiapkan melalui metode maserasi etanol, dimasukkan ke dalam nanoemulsi menggunakan ultrasonikasi, dan kemudian diformulasikan

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menjadi gel berbahan dasar karbopol. Nanoemulgel tersebut dievaluasi sifat fisikokimianya dan diuji terhadap MRSA menggunakan metode mikrodilusi, MIC, dan MBC.
Hasil penelitian menunjukkan bahwa ekstrak tunggal Curcuma zedoaria dan Curcuma xanthorrhiza memiliki aktivitas antibakteri yang lebih kuat terhadap MRSA dibandingkan dengan formulasi kombinasinya. Nanoemulgel tersebut juga menunjukkan karakteristik fisikokimia yang stabil dan sesuai untuk aplikasi topikal.
Secara keseluruhan, nanoemulgel kombinasi dua jenis Curcuma ini menunjukkan potensi yang menjanjikan sebagai pendekatan terapeutik berbasis bahan alam untuk mengatasi infeksi MRSA, sekaligus menjembatani kearifan herbal tradisional dengan nanoteknologi modern.

***Kata kunci**: Nanoemulgel, Curcuma xanthorrhiza, Curcuma zedoaria, MRSA, sinergi antibakteriethanol*

INTRODUCTION

Antibiotic resistance remains a critical global health threat, with the World Health Organization warning of a potential "post-antibiotic era" in which routine infections become increasingly difficult to treat (Talebi, et al. 2019; Chandra, et al, 2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most challenging pathogens in this context, causing a wide spectrum of infections and exhibiting resistance to multiple classes of antibiotics (Gajdács, et al. 2019; Mancuso, et al, 2021; Mahjabeen, et al, 2022). These limitations highlight the need for new antibacterial agents, including those derived from natural products.

Medicinal plants continue to receive attention for their diverse bioactive compounds and potential antibacterial activity (Chaachouay, et al. 2024; Manisha, et al. 2025). In Indonesia, *Curcuma xanthorrhiza* and *Curcuma zedoaria* are traditionally used medicinal plants known for their curcuminoids and sesquiterpenes, which have demonstrated antimicrobial and anti-inflammatory effects (Subositi, et al. 2019; Simamora, et al. 2024). While combining plant extracts is often proposed to enhance activity through synergistic interactions, such effects are not guaranteed and may result instead in neutral or antagonistic outcomes (Cheesman, et al. 2017; Coates, et al. 2020; Jubair, et al. 2021). Experimental evaluation is therefore required to determine whether combinations provide any advantage over single extracts.

Nanotechnology offers opportunities to improve the solubility, stability, and topical delivery of phytochemicals. Nanoemulgels, which incorporate nanoemulsion droplets into a gel matrix, enhance penetration and support controlled release, thereby improving the therapeutic performance of herbal preparations (Rahman, et al. 2020; Preeti, et al 2023; Ansari, et al 2025). Prior studies indicate that nanoemulgel systems can increase antibacterial efficacy compared to conventional formulations (Pramuningtyas, et al. 2024; Eid, et al 2021; Ibrahim, et al. 2024).

Although the antibacterial activities of *C. xanthorrhiza* and *C. zedoaria* have been independently reported, limited data are available comparing their single and combined use within nanoemulgel systems (Giri, et al 2024; Bagheri, et al. 2024). Understanding these interactions is essential for guiding rational formulation development.

This study aimed to develop nanoemulgel formulations containing *C. xanthorrhiza*, *C. zedoaria*, and their combination, and to evaluate their antibacterial activity against MRSA. The findings provide insight into whether combining these extracts enhances antibacterial potency or whether single-extract formulations remain superior.

MATERIALS AND METHODS

Materials

Materials such as tetracyclin, phosphate buffer saline (PBS), tablets of crystal violet, anhydrous sodium sulfate, dimethyl sulfide (DMSO), and microbial agar were purchased from several notorious pharmaceutical companies such as Sigma-Aldrich, Merck, Becton, and Dickinson for analytical purposes.

Bacterial Strains

The Methicillin-resistant *Staphylococcus aureus* strains were collected from several clinical isolates conducted by Cendekia Laboratorium Medan, Indonesia. The strain was stored in a specialized bacterial storage refrigerator added with glycerol stock at −80 ˚C and re-grown in BHI agar at 37 ˚C for 18 – 20 hours before being used for analysis in this study.

Extracts Preparation

In a glass container, 2500 mL of 96% ethanol was macerated with 250 g of Simplicia powder. First, the rim of the macerated container was filled with 75% solvent. The mixture was allowed to sit for 24 hours with stirring. The procedure was then continued. The macerate was then collected in a container and the remaining 25% of the solvent was added. Maceration was performed until day seven. To obtain a more concentrated ethanol extract, macerate products were collected and concentrated using rotary evaporators.

Chemical Compositions Analysis of *C. xanthorrhiza and C. zedoaria* extract

The extract exhibiting the most potent antibacterial activity was further analyzed using LC-MS/MS to determine its chemical composition (Mangurana, et al. 2019). Briefly, the data were collected and determined by referring to the Wiralab Analitika Solusindo dataset to obtain the peak area of each chromatogram for identification and calculation.

Formulation Nanoemulgel

The preparation of the nanoemulgel began with the development of a nanoemulsion system. To start, 0.5 grams of the ethanolic plant extract was dissolved in 5 mL of virgin coconut oil, which served as the carrier oil. This oily extract solution was then combined with Tween 80 (as surfactant) and PEG 400 (as co-surfactant) in a 3:1 ratio, ensuring the stability of the emulsion system. The oil phase and the surfactant–cosurfactant mixture were slowly introduced into distilled water drop by drop while undergoing homogenization. Sonication was carried out at a frequency of 20 kHz for 15 minutes to reduce droplet size and enhance stability. This process resulted in a nanoemulsion with particle sizes ranging between 20–200 nm, confirmed using a Particle Size Analyzer (PSA).

In the next stage, the gel base was prepared. Carbopol 940 (1% w/v) was dispersed in distilled water and stirred with a magnetic stirrer until a uniform gel matrix was formed. Neutralization with triethanolamine (0.5%) was performed to adjust the pH to 6–7, making the gel suitable for skin application. The nanoemulsion was then gradually incorporated into this gel base with continuous stirring, resulting in a stable and homogenous nanoemulgel formulation (Kesharwani, et al. 2020).

To evaluate the effect of different concentrations and combinations of extracts, several formulations were designed. For the single-extract nanoemulgels: **Formula A:** 5% *C. xanthorrhiza* (temulawak) extract; **Formula B:** 10% *C. xanthorrhiza* extract; **Formula C:** 20% *C. xanthorrhiza* extract; **Formula D:** 5% *C. zedoaria* (temu putih) extract; **Formula E:** 10% *C. zedoaria* extract; **Formula F:** 20%

C. zedoaria extract. For the combination formulations: **Formula G:** 10% *C. xanthorrhiza* + 10% *C. zedoaria* (total 20%); **Formula H:** 20% *C. xanthorrhiza* + 20% *C. zedoaria* (total 40%)

This systematic variation allowed a clear comparison between single-plant and combined formulations, making it possible to evaluate not only the effect of concentration but also the potential synergistic antibacterial activity of the two Curcuma species when used together.

Determination Antibacterial Activity

Antibacterial activity was measured using the microdilution method, with several modifications to previous studies (Hasibuan, et al 2021; Nandhakumar, et al 2023), to get the minimal inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. The MIC was determined in accordance with Clinical Laboratory Standard Institute Guidelines M7-A6 (2008). Bacterial suspension was generated on BHI broth (BHIB) and adjusted to 0.5 McFarland standard (- 1.5 x 10^8 CFU/mL), which was then diluted with 0.9% NaCl sterile solution to get 1.5 x 10^6 CFU/mL bacterial suspension. To begin, 100 µL of BHI broth was added to each well. The test sample was then added and diluted with the mix solution using the two-fold dilution procedure to achieve serial dilutions ranging from 500 µg/mL to 3.9 µg/mL. Next, 100 µL of the previously prepared bacterial solution was applied to each well. Tetracycline solution was employed as a comparison, while DMSO 1% v/v served as a negative control. Microplates were incubated at 37 ˚C for 24 hours. The MIC value is shown by the minimum concentration that resulted in a clear solution without precipitation in each well. MBC is the lowest dose of an antimicrobial agent that inhibits bacterial growth on BHI agar media. The experiment involved putting colonies at MIC concentrations into a petri dish containing solid media and incubating for 24 hours at 37 ˚C. After incubation, the bactericidal concentration was calculated using the least concentration that resulted in no microbial growth in the petri dish.

Checkerboard Assay

This test was conducted using the checkerboard method (Sharma, et al. 2023; Odds, 2003) with several modifications. *Curcuma xanthorrhiza* extract was diluted with BHI broth media using a two-fold dilution along the x-axis of 96-well plates. The Tetracycline was then made by twofold dilution along the y-axis. MRSA suspension (about 1×10^6) was added to each well and incubated at 37°C for 24 hours. Following incubation, the fractional inhibitory concentration index (FICI) was determined using the formula below to investigate the interaction of *Curcuma xanthorrhiza* extract with Tetracycline.

$$\text{FICI} = \frac{\text{MIC of extract in combination}}{\text{MIC of extract alone}} + \frac{\text{MIC of vancomycin in combination}}{\text{MIC of vancomycin alone}}$$

The index was categorized into synergistic (FICI ≤ 0.5), additive (0.5 ≤ FICI ≤ 1), indifferent (1 ≤ FICI ≤ 4), and antagonistic (FICI > 4)

Anti-biofilm Formation Assay

Antibiofilm formation was tested using 96-well microplates (Sharma, et al. 2023). Briefly, the wells were filled with the sample and bacterial suspension, then incubated at 37 °C for 24-48 hours, while the control consisted of untreated wells. After one day, the wells were rinsed with PBS and incubated with 200 ml of crystal violet (0.3% in DMSO) added selectively. The degradation of biofilm was subsequently measured at 560 nm.

Bacteriolysis Activity

A bacteriolysis test was conducted as described in a previous study (Sharma, et al. 2023; Septama, et al. 2018). Bacteriolytic activity is related to the extract's ability to alter the permeability of bacterial cell membranes, which is measured by determining the maximum absorption of intracellular material out of the cell due to damaged cell membrane integrity, as well as the reuptake of crystal violet, which is used as a cell membrane indicator. The dose of tested samples was based on the results of the MIC and checkerboard assay. The bacterial solution was produced in normal saline to have an optical density (OD) of around 0.4 at 590 nm. 500 µL of sample solution was added to an Eppendorf Tube containing 125 µg/mL *Curcuma xanthorrhiza* extract, 7.815 µg/mL Tetracycline, and 3.9 µg/mL *Curcuma xanthorrhiza* extract and 1.915 µg/mL Tetracycline, respectively. The prepared bacterial suspension (500 µL) was added to the tubes and centrifuged at 13000 rpm for 1 hour. Furthermore, the supernatant was transferred to a microplate to determine OD260.

This research also seeks to quantify the absorbance of nucleic acids that leak from the bacterial cell membrane. In addition, a crystal violet absorption assay was used to establish the effect of *Curcuma xanthorrhiza* extract on membrane instability. The precipitate from the previous treatment was mixed with 500 µL of 0.001% crystal violet. Afterward, the tube was centrifuged at 13000 rpm for 15 minutes. The OD590 of the supernatant was measured to evaluate the absorption of crystal violet that was not bound to the bacterial cell membrane, or free crystal violet.

RESULTS AND DISCUSSION

The Zingiberaceae family of plant *Curcuma xanthorrhiza*, according to the results of plant identification conducted at the Medanense Herbarium Plant Systematics Laboratory (MEDA) Number: 1842/MEDA/2025. **Table 1** displays the results of the extract yield from *Curcuma xanthorrhiza* and *Curcuma zedoaria* extract.

Table 1. Extraction rendement

Sample	Sample Weight (g)	Extract Weight (g)	Yield (%)	FHI Requirements (%)
Curcuma xanthorrhiza	382	136	35.60	Not less than 18
Curcuma zedoaria	504	182	36.11	Not less than 19

The *Curcuma xanthorrhiza* and *Curcuma zedoaria* extracts were found to contain secondary metabolite compounds such as flavonoids, phenols, glycosides, and steroids/triterpenoids based on the phytochemical screening results presented in Table 2. Based on the antibacterial activity evaluation, *Curcuma zedoaria* extract, which exhibited the most potent antibacterial effect, was further analyzed using Liquid Chromatography–Mass Spectrometry (LC-MS/MS) to characterize its phytochemical composition. The detected chromatographic peaks were compared with MS databases to identify the compounds present. The LC-MS/MS chromatogram of the *Curcuma zedoaria* extract is shown in Figure 1.

Table 2. Results of phytochemical screening of *Curcuma xanthorrhiza* and *Curcuma zedoaria* extract from the Rutaceae Family

No.	Content	*Curcuma xanthorrhiza* extract	*Curcuma zedoaria* extract
1.	Flavonoids	+	+
2.	Alkaloids	+	+
3.	Saponins	-	-
4.	Phenols	+	+
5.	Glycosides	+	+
6.	Steroids/terpenoid	+	+

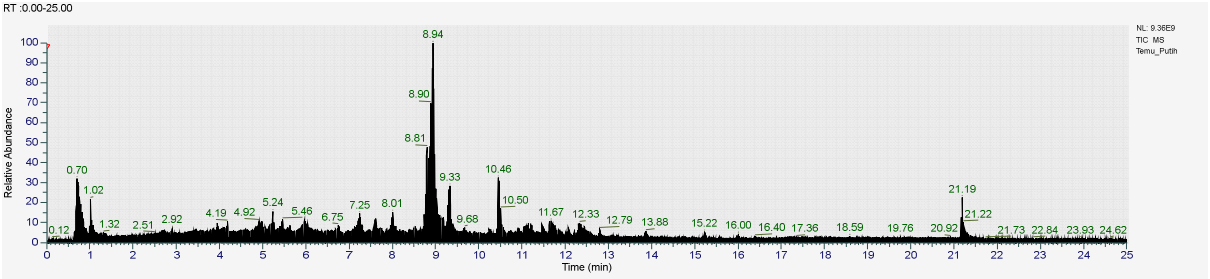


Figure 1. LC-MS/MS Spectrum of *Curcuma zedoaria* extract

Based on the above LC-MS/MS analysis of the *Curcuma zedoaria* extract, it is known that the compound components in the *Curcuma zedoaria* extract are 50 compounds identified, with the main constituents consisting of coumarin, flavonoids, glycosides, terpenoids, fatty acids, shown in **Table 3**. These results are also supported by previous researchers who reported that *Curcuma zedoaria* extract was found to contain flavonoids, phenolics, terpenoids, curcuminoid compounds, curcumenolactone A curzerenone, curcumene (Ab Halim, et al. 2012). Curcuminoid are secondary metabolites that are most abundant in curcuma species (Sun, et al. 2017). The antibacterial activity of curcuminoids is seen through the mechanism of damage/modification of bacterial membrane structures, production or secretion of toxins, and prevention of biofilm formation (Olchowik-Grabarek, et al. 2020; Zheng, et al. 2020).

Table 3. Chemical Constituents of *Curcuma zedoaria* extract identified by LC-MS/MS

No	**Name**	**Formula**	**Calc. MW**	**m/z**	**RT [min]**
1	Choline	C5 H13 N O	103.09957	104.10685	0.701
2	Isoleucine	C6 H13 N O2	131.09448	132.10176	1.019
3	Crotonic acid	C4 H6 O2	86.03669	87.04391	1.025
4	Adenine	C5 H5 N5	135.05443	136.06158	0.798
5	Adenosine	C10 H13 N5 O4	267.09645	268.10373	1.018
6	L-Tyrosine	C9 H11 N O3	181.0737	182.08098	1.017
7	4-Hydroxy-6-methyl-2-pyrone	C6 H6 O3	126.03168	125.0244	2.405

No	Name	Formula	Calc. MW	m/z	RT [min]
8	Methylsuccinic acid	C5 H8 O4	132.04219	131.03491	1.657
9	Gluconic acid	C6 H12 O7	196.05826	195.05098	0.755
10	NP-016141	C22 H29 N O3	355.21446	356.22174	11.854
11	Salicylic acid	C7 H6 O3	138.03169	137.02441	2.617
12	NP-020521	C18 H32 O3	296.23503	295.22775	10.939
13	DL-Arginine	C6 H14 N4 O2	174.11144	175.11871	0.624
14	Valine	C5 H11 N O2	117.0788	118.08608	0.713
15	Azelaic acid	C9 H16 O4	188.10482	187.09755	4.866
16	Linoleoyl ethanolamide	C20 H37 N O2	323.28215	324.28943	11.876
17	(+/-)9,10-dihydroxy-12Z-octadecenoic acid	C18 H34 O4	314.24565	313.23837	9.362
18	NP-008274	C15 H20 O4	264.13597	265.14325	5.963
19	L-Phenylalanine	C9 H11 N O2	165.07874	166.08601	0.746
20	Oleoyl ethanolamide	C20 H39 N O2	325.29787	326.30515	12.518
21	NP-021797	C12 H22 O3	214.15686	213.14958	8.732
22	NP-018711	C15 H20 O4	264.13594	265.14316	7.238
23	α,α-Trehalose	C12 H22 O11	342.11537	387.11359	0.79
24	6-Hydroxycaproic acid	C6 H12 O3	132.0786	131.07133	3.252
25	NP-000510	C21 H25 N O3	339.18324	340.19049	11.145
26	6-Methylquinoline	C10 H9 N	143.07337	144.08064	2.723
27	α-Pinene-2-oxide	C10 H16 O	152.12003	153.1273	3.409
28	(2E,4E)-N-(2-methylpropyl)deca-2,4-dienamide	C14 H25 N O	223.19347	224.20074	10.546
29	(±)9-HpODE	C18 H32 O4	312.22995	311.22269	9.622
30	NP-018534	C15 H22 O4	266.15162	267.15891	4.186
31	Gentisic acid	C7 H6 O4	154.02646	153.01918	1.947
32	Hexadecanamide	C16 H33 N O	255.25597	256.26324	14.25
33	(6E)-7-(2H-1,3-benzodioxol-5-yl)-1-(piperidin-1-yl)hept-6-en-1-one	C19 H25 N O3	315.18306	316.19034	10.449
34	NP-020078	C17 H28 O3	280.20381	279.19653	10.473

No	Name	Formula	Calc. MW	m/z	RT [min]
35	trans-Aconitic acid	C6 H6 O6	174.01634	173.00906	1.018
36	NP-002676	C15 H26 O4	270.18309	269.17581	7.721
37	Deoxylimonin	C26 H30 O7	454.1971	455.20438	8.472
38	16-Hydroxyhexadecanoic acid	C16 H32 O3	272.23506	271.22778	12.336
39	3-Hydroxybutyric acid	C4 H8 O3	104.04728	103.04	1.157
40	(-)-Caryophyllene oxide	C15 H24 O	220.18263	221.1899	9.135
41	8-hydroxy-11-(hydroxymethyl)-1,5,11-trimethyltricyclo[6.2.1.0Â?,â ¶]undec-2-en-9-one	C15 H22 O3	250.15669	251.16397	5.438
42	NP-008309	C11 H13 N O3	207.08955	206.08228	4.175
43	9-hydroxy-2,10,10-trimethyltricyclo[6.3.0.0Âa,â µ]undec-6-ene-6-carboxylic acid	C15 H22 O3	250.15664	251.16393	6.029
44	11-Ketotestosterone	C19 H26 O3	302.188	303.19531	9.15
45	(1S,2S,4R,7E,11S)-4,8-dimethyl-12-methylidene-3,14-dioxatricyclo[9.3.0.0Â?,â ´]tetradec-7-en-13-one	C15 H20 O3	248.141	249.14827	8.008
46	2-Methylglutaric acid	C6 H10 O4	146.05783	145.05055	2.186
47	NP-008515	C15 H20 O4	264.13595	265.14322	8.196
48	Oleoyl-L-α-lysophosphatidic acid	C21 H41 O7 P	436.25849	435.25107	11.901
49	NP-022500	C15 H18 O4	262.12029	263.12756	7.27
50	Suberic acid	C8 H14 O4	174.08914	173.08186	3.968

Table 4 shows the Minimum Inhibitory Concentration (MIC) values, which represent the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation. The lower the MIC value, the more effective the compound was in inhibiting bacterial growth. The minimum bactericidal concentration (MBC) was determined by subculturing the MIC from the microdilution plate onto Brain Heart Infusion Agar (BHIA) shown in **Table 4**.

Table 4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values of Nanoemulgel *Curcuma xanthorrhiza* and *Curcuma zedoaria* extract, and positive control

No.	Formula	Composition	MIC (µg/mL)	MBC (µg/mL)
1.	A	5% C. zedoaria	15.625	31.25

2.	B	10% C. zedoaria	31.25	62.50
3.	C	20% C. zedoaria	15.625	31.25
4.	D	5% C. xanthorrhiza	15.625	31.25
5.	E	10% C. xanthorrhiza	15,625	31.25
6.	F	20% C. xanthorrhiza	31.25	62.50
7.	G	10% C. xanthorrhiza + 10% C. zedoaria (total 10%)	125	250
8.	H	20% C. xanthorrhiza + 20% C. zedoaria (total 40%)	250	500
9.	T	Tetracycline (positive control)	7.8125	15,625

The checkerboard assay demonstrated a synergistic interaction between *Curcuma zedoaria* extract (Formula A) and tetracycline, as indicated by an FIC index of 0.50, while the combination of *Curcuma xanthorrhiza* extract (Formula E) with tetracycline showed an additive effect (FIC = 0.75). Components of *Zingiberaceae* extracts are known to interact with bacterial membranes, leading to cytoplasmic leakage and alterations in ion transport, which may enhance antibiotic penetration (Voravuthikunchai, 2007). These mechanisms likely contribute to the improved antibacterial activity observed when the extracts were combined with tetracycline (Table 5)

Table 5. The Result of the Checkerboard Test Between Nanomulgel Formula A ; Formula E and Tetracycline

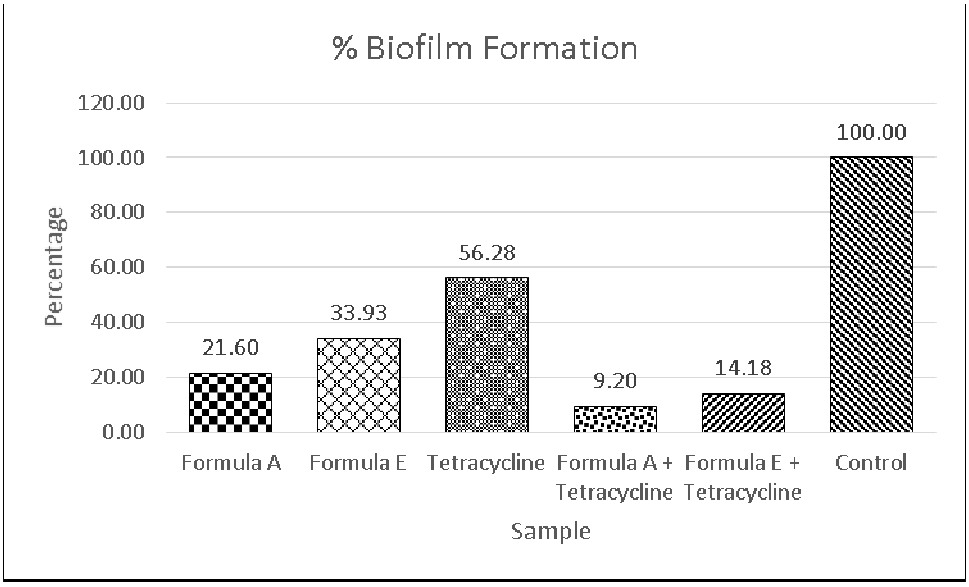
No	Formula	MICa (µg/mL)	MICc (µg/mL)	FIC index	Interpretation
1	A + T	15.625/ 7.8125	3.906/ 1.953	0.50	Synergistic
2	E + T	15.625/ 7.8125	7.8125/ 1.953	0.75	Aditif

Note: a = alone; c = combination
A = 5% C. zedoaria
E = 10% C. xanthorrhiza
T = Tetracycline (positive control)

The results of this synergistic effect test were influenced by several contributing factors, such as differences in the targets of action of the secondary metabolites and Tetracycline. Secondary metabolite components are known to have targeted actions by changing the permeability of bacterial cell membranes, damaging cell walls, damaging bacterial membrane proteins, coagulating cytoplasm, hydrolyzing ATP synthesis in membranes, and reducing bacterial proton pump activity (Alam, et al. 2022; Yu, et al. 2020). Therefore, it can be said that this combination of secondary metabolites can synergize with the mechanism of destroying bacterial membranes and cell walls to help Tetracycline inhibit bacteria in the early stages of cell wall peptidoglycan synthesis (Zhou, et al. 2022). Therefore, it can be concluded that with the different targets of action of secondary metabolites and Tetracycline, both can synergistically inhibit the growth of MRSA, as indicated by the FIC Index values obtained.

Microorganisms are capable of forming biofilms (Figueiredo, et al. 2008; Rios, et al. 2016). As shown in **Figure 2**, both *Curcuma zedoaria* (Formula A) and *Curcuma xanthorrhiza* (Formula E) extracts

reduced biofilm formation compared with the control; however, their combinations with tetracycline exhibited markedly stronger inhibition. The combination of Formula A with tetracycline reduced biofilm formation to 9.20% and Formula E with tetracycline to 14.18%, which is substantially lower than the extracts or tetracycline used individually. Previous studies support these findings, indicating that limonoids can disrupt quorum-sensing signaling and inhibit biofilm development (Rios, et al. 2016; Fournière, et al. 2020). **Figure 3** shows that the combinations of the nanoemulgel extracts with tetracycline produced the highest OD260 values, indicating substantially greater MRSA cell leakage compared with individual treatments. This enhanced leakage aligns with previous findings suggesting that plant extracts containing multiple bioactive compounds can disrupt membrane structure and compromise membrane integrity (Ambrosio, et al. 2019; Bakkali, et al. 2008). According to the results, the combinations of the nanoemulgel extracts with tetracycline caused the highest crystal violet uptake, reaching 95.45% for Formula A + tetracycline and 90.92% for Formula E + tetracycline, which indicates substantially greater membrane permeability than either extract or tetracycline alone (**Figure 4**). The pronounced increase in crystal violet absorption is likely associated with the broader range of bioactive compounds in the extracts that can disrupt membrane integrity and facilitate increased dye penetration (Lamut, et al. 2019).



Note: : A = 5% C. zedoaria

E = 10% C. xanthorrhiza

T = Tetracycline ; Control = MRSA

Figure 2. The Effect of Nanoemulgel Combination extract and Tetracycline in Biofilm Degradation. *P<0.05 Significant Different with Respective Control

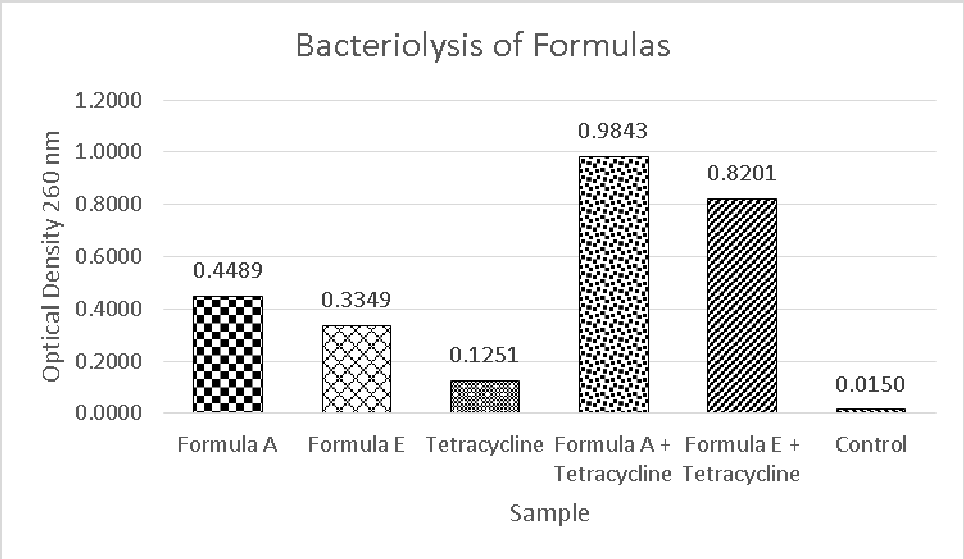


Figure 3. Optical Density (OD) 260 nm MRSA Treated with Nanoemulgel Combination extract and Tetracycline. *P<0.05 Significant Different with Respective Control

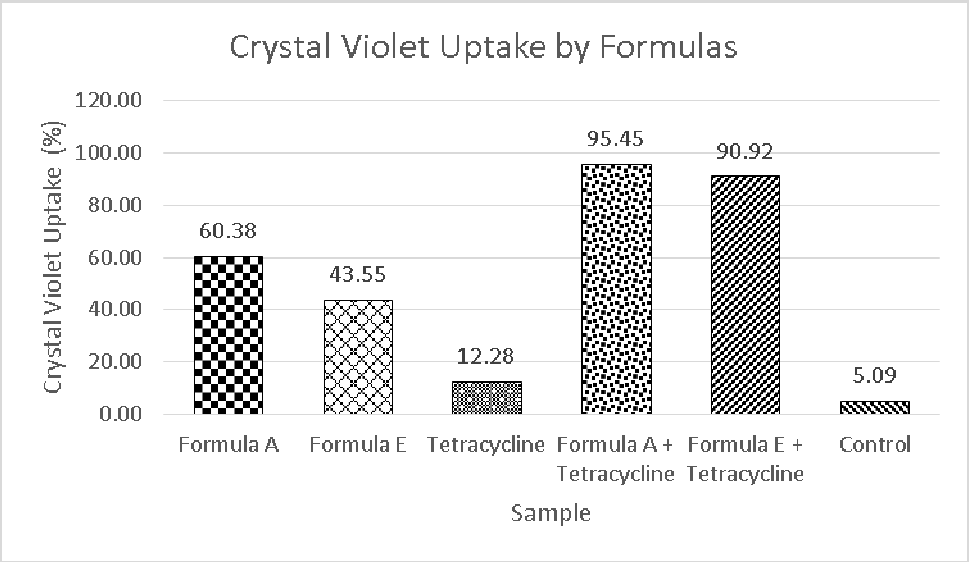


Figure 4. The Uptake of Crystal Violet by MRSA after Treatment with Nanoemulgel Combination extract and Tetracycline. *P<0.05 Significant Different with Respective Control

CONCLUSION

Nanoemulgel formulations containing Curcuma xanthorrhiza and Curcuma zedoaria extracts demonstrate antibacterial activity against MRSA, with individual extracts showing membrane-disruptive, biofilm-inhibitory, and permeability-enhancing effects. Although combinations of the two extracts alone do not improve antibacterial activity, their combinations with tetracycline exhibit synergistic or additive interactions, reflected by stronger biofilm inhibition, extensive membrane leakage, and markedly increased crystal violet uptake. These findings indicate that the extracts enhance antibiotic penetration and may modulate resistance-related mechanisms such as membrane integrity and permeability. Therefore, further in

vivo studies are required to evaluate the bioavailability, therapeutic efficacy, and safety of nanoemulgel formulations combining these extracts with conventional antibiotics.

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