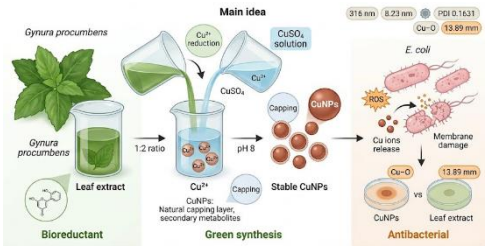
JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA), Vol. 11, No. 1, 2026 pp. 76-90
Chemistry Education Study Program, Universitas Sebelas Maret ISSN 2503-4146
https://jurnal.uns.ac.id/jkpk ISSN 2503-4154 (online)

Green Synthesis of Copper Nanoparticles Using Gynura procumbens Leaf Extract as a Bioreductant and Their Antibacterial Activity Against *Escherichia coli*

Nancy Rivana[1*], Suyatno Sutoyo[1], Radita Yuniar Arizandy[2], Wahyu Setyarini[2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia*
[2]*Lembaga Penyakit Tropis, Universitas Airlangga, Surabaya, Indonesia*

ABSTRACT



Copper nanoparticles (CuNPs) are nanomaterials extensively utilized in optics, electronics, biology, catalysis, and as antibacterial agents. Sustainable synthesis approaches employing plant-derived bioreductants have garnered significant attention. In this study, CuNPs were synthesized via green chemistry using Gynura procumbens leaf extract as a bioreductant. Synthesis was performed by combining the leaf extract with 0.1 M $CuSO_4$ solution at volume ratios of 1:1, 1:2, 1:3, and 1:4. The mixture with the optimal volume ratio was further optimized at pH values of 8, 9, 10, and 11. The resulting CuNPs were characterized by UV-Vis spectroscopy, FTIR, and PSA. Antibacterial activity against Escherichia coli (ATCC 25922) was evaluated using the disk diffusion method. Optimal synthesis was achieved at a 1:2 volume ratio and pH 8. The CuNPs exhibited a maximum UV-Vis absorption at 316 nm, an average particle size of 8.23 nm, and a polydispersity index of 0.1631. FTIR spectra confirmed Cu–O bond vibrations at 457.64 and 617.14 cm-1. The synthesized CuNPs demonstrated strong antibacterial activity against E. coli, with an inhibition zone diameter of 13.89 ± 0.45 mm. These findings suggest that CuNPs synthesized via green methods using G. procumbens extract as a bioreductant have potential as antibacterial agents, particularly against E. coli.

Keywords: *Green Synthesis, Antibacterial Activity, Bioreductant, Copper Nanoparticles, Gynura procumbens*

**Corresponding Author: nancy.22105@mhs.unesa.ac.id*

How to cite: N. Rivana, S. Sutoyo, R.Y. Arizandy, and W. Setyarini, " Green Synthesis of Copper Nanoparticles Using Gynura procumbens Leaf Extract as a Bioreductant and Their Antibacterial Activity Against Escherichia coli," *Jurnal Kimia dan Pendidikan Kimia (JKPK)*, vol. 11, no. 1, pp.76-90, 2026. [Online]. Available: https://doi.org/10.20961/jkpk.v11i1.116736
Received: 2026-03-15 *Accepted: 2026-04-20* *Published: 2026-04-30*

INTRODUCTION

Copper nanoparticles (CuNPs) have gained significant attention due to their extensive applications across various fields, including health, electronics, cosmetics, and catalysis [1][2][3][4][5]. the development of copper nanoparticles (CuNPs) remains challenged by the toxicity of their conventional synthesis. Traditional bottom-up chemical methods rely on toxic reductants such as $NaBH_4$, which lead to significant environmental pollution [6]. To overcome this, recent studies emphasize green synthesis, a biocompatible and cost-effective approach using biological organisms or plant extracts as bioreductants under mild conditions [7][8]. Building upon this eco-friendly approach, the current study specifically investigate *Gynura procumbens leaf extract* to synthesize CuNPs, addressing the specific need for antibacterial activity.

Plant extracts serve as bioreductants due to secondary metabolites such as flavonoids and polyphenols, which act as both bioreductants and capping agents [9]. Among various medicinal plants, *G. procumbens* stands out as a highly promising and distinct candidate. Previous studies indicate that *G. procumbens* leaf extract demonstrates exceptional antioxidant activity. This is shown by a very strong IC_{50} value of 15.01 mg/L [10]. Such potent antioxidant capacity suggests superior reducing power compared to other conventional plant extracts. This makes it highly suitable for facilitating rapid, stable nanoparticle synthesis. In this study, copper was selected due to its extensive applications in optical, electronic, biological, catalytic, and antibacterial fields [11][12]. Additionally, copper has attracted attention for its ease of availability and economic viability compared to more expensive noble metals such as silver, gold, and platinum [13].

Gynura procumbens plant belongs to the Asteraceae family and is widely known as a traditional medicine used to treat various diseases, including cancer, diabetes mellitus, dengue fever, kidney disease, skin rashes, inflammation, and hypertension, as well as for its antibacterial properties [14]. This plant has the potential to serve as a bioreductant in the synthesis of copper nanoparticles, as it contains phenolic compounds, specifically flavonoids [10][15]. Quercetin is a flavonoid-type secondary metabolite present in *G. procumbens* leaves, containing phenolic hydroxyl groups capable of reducing copper ions into copper nanoparticles [16][17]. Moreover, these compounds can act as capping agents, stabilizing the synthesized CuNPs [16].

While CuNPs have been synthesized using bioreductants from various plant extracts, as *Ephedra Alata* [18], *Morinda citrifolia* [19], *Gloriosa superba* [20], *Myristica Fragrans* [21], dan *Carica pubescens* [22]. However, no studies had reported using *G. procumbens*. This study introduces a novel bioreductant. It also addresses a critical research gap by systematically optimizing the extract-to-$CuSO_4$ precursor volume ratio and synthesis pH. Optimizing the volume ratio is crucial for establishing the ideal composition. This ensures that the reduction reaction proceeds to completion while preventing particle agglomeration [23]. At the same time, an optimal pH facilitates the deprotonation of phenolic compounds in the extract. This enhances their electron-donating capacity, allowing efficient reduction of copper ions and robust capping stabilization [24]. As a result, this optimization directs the formation of smaller, highly stable CuNPs with a maximized surface-area-to-volume ratio. Ultimately, this delivers a potent antibacterial performance to combat drug-resistant bacteria globally.

Following synthesis optimization, this study employs *Escherichia coli* as the bacterial model to evaluate the antibacterial potential of the CuNPs. *E. coli* is a major Gram-negative pathogen. It causes severe gastrointestinal infections and contributes to nearly 1.7 billion childhood diarrheal cases each year. It also leads to over 490,000 deaths annually among children under 9 years old [25]. The CuNPs synthesized by *G. procumbens* are expected to exhibit potent

antibacterial activity. They generate reactive oxygen species (ROS) and release copper ions. This mechanism triggers targeted oxidative stress, disrupts the cell membrane, and degrades intracellular targets [12]. Despite the critical need for such treatments, the use of *G. procumbens*-mediated CuNPs against *E. coli* has not been explored. This study was conducted to evaluate the potential of *G. procumbens* leaf extract as a bioreductant for the synthesis of CuNPs by optimizing the extract-to-$CuSO_4$ volume ratio and pH, followed by evaluation of the antibacterial activity of the synthesized CuNPs against *E. coli*. These two parameters are expected to maximize reducing and capping capacities of extract, resulting in highly stable and optimally sized CuNPs. Establishing this optimized green-synthesis approach is anticipated to provide a novel, cost-effective, and offering a promising alternative solution to combat bacterial infections.

METHODS

1. Material

The materials used included G. procumbens leaves obtained from the Herbal Laboratory of Materia Medica (East Java Province) with a voucher specimen 240315.SMY.6.MMB.001, $CuSO_4.5H_2O$ (Merck), NaOH (Merck), Mueller Hinton Agar (Merck), NaCl (Merck), Escherichia coli (ATCC 25922), filter paper (Whatman No. 42), sterile paper disks (MN Germany), ciprofloxacin (Sigma-Aldrich), and double-distilled water (DDW) (Otsuka).

2. Preparation of G. procumbens Leaf Extract

A total of 10 grams of G. procumbens leaf powder was added to 200 mL of double-distilled water. The mixture was covered with aluminum foil to prevent light exposure and heated at 65°C while stirring with a magnetic stirrer (DLAB MS7-H550) for 30 minutes. The mixture was allowed to cool to room temperature for approximately 1 hour then filtered using Whatman No. 42 filter paper to obtain G. procumbens leaf extract, which was ready to be used as a bioreductant Extract was stored in a dark environment at 4°C and used within 72 hours to maintain its stability as a bioreductant [26].

3. Synthesis of Copper Nanoparticles (CuNPs)

The *G. procumbens* leaf extract was added to a 0.1 M $CuSO_4$ precursor solution at volume ratios of 1:1, 1:2, 1:3, and 1:4, with a total volume of 60 mL. This volume ratio was chosen based on established literature for green-synthesized copper nanoparticles, where varying the bioreductant-to-precursor ratio is critical for controlling particle size and stability [23][27][28]. The mixture was stirred using a magnetic stirrer at 1500 rpm for 90 minutes at 60°C to accelerate the reaction rate. The mixture was left to stand for 24 hours, resulting in a dark green color. This color shift was recorded as a preliminary indication of the reduction of copper ions and the potential formation of CuNPs [29].

Each mixture was characterized using a UV-Vis spectrophotometer (Shimadzu UV-Vis 1800) at a wavelength of 200-800 nm to determine the optimal volume ratio and to provide definitive evidence of CuNP formation. The mixture with the optimum volume ratio was then subjected to

pH optimization at pH 8, 9, 10, and 11 by adding 0.1 M NaOH. The solution was stirred with a magnetic stirrer at 1500 rpm for 90 minutes, and the maximum wavelength was measured using a UV-Vis spectrophotometer to determine the optimum pH [24]

The synthesized mixture with the optimum volume ratio and pH was centrifuged at 5000 rpm for 30 minutes; the obtained CuNPs precipitate was separated from the filtrate by decantation, washed with double-distilled water, and then dried using a freeze-dryer (Martin Christ Alpha 1-2) [28]. The obtained CuNPs powder was characterized using FTIR (Shimadzu IR Tracer-100) and PSA (BIOBASE BK-802N), and its antibacterial activity against *E. coli* was evaluated.

4. Antibacterial Activity Assay

The antibacterial activity of the synthesized CuNPs was evaluated against the Gram-negative bacterium *E. coli* using the disk diffusion method. First, the bacterial suspension was standardized to a turbidity equivalent to the 0.5 McFarland standard. Then, the bacterial inoculum was spread onto solid Mueller-Hinton agar media. Afterward, sterile paper disks soaked in the CuNPs colloid were placed on the agar surface.

After 24 hours of incubation at 36°C, the diameter of the bacterial growth inhibition zone was measured with a caliper. To ensure consistency, the same procedure was applied to the *G. procumbens* leaf extract, ciprofloxacin (100 ppm) as the positive control, and double-distilled water as the negative control. All test treatments were carried out in triplicate and presented as Mean ± Standard Deviation (SD) [30][31]. Subsequently, the antibacterial activity was classified into four categories: weak, moderate, strong, and very strong, corresponding to inhibition zone diameters of <5 mm, 5-10 mm, 10-20 mm, and >20 mm, respectively [32]. To determine whether there were significant differences in values between CUNPs and plant extracts, either an Independent-T-test or a Kruskal-Wallis test was applied, depending on the normality of the antibacterial activity data. All statistical analyses were performed using SPSS Statistics ver. 26, with the level of significance set at $p < 0.01$ [33].

RESULT AND DISCUSSION

1. Optimization of Composition in Synthesis CuNPs and the UV-Vis Spectroscopy Analysis

In this study, CuNPs were synthesized by mixing *G. procumbens* leaf extract with 0.1 M $CuSO_4$ solution at volume ratios of 1:1, 1:2, 1:3, and 1:4. The reaction mixture was analyzed using UV-Vis spectroscopy, and the results are presented in Table 1 and Figure 1. Based on the data, CuNP formation was observed in all compositions, as indicated by the maximum absorption wavelengths in the range of 300–400 nm [34]. The formation is further supported by comparing the UV-Vis spectra of the CuNPs, $CuSO_4$, and *G. procumbens* extract in Figure 2. The UV-Vis spectra of the $CuSO_4$ solution and the *G. procumbens* leaf extract exhibited maximum absorption wavelengths at 237 and 326 nm, respectively. The reaction between the $CuSO_4$ precursor and the *G. procumbens* leaf extract produced copper nanoparticles with a

peak at 316 nm, distinct from both reactants. The disappearance of the absorption peaks of $CuSO_4$ and *G. procumbens* leaf extract in the UV-Vis spectrum of the copper nanoparticles indicates that both reactants had successfully reacted to produce CuNPs

Table 1. The maximum absorption wavelength and absorbance of synthesized CuNPs at variation of volume ratio (composition)

Composition	λmax (nm)	Absorbance
1 : 1	322	0.241
1 : 2	316	0.498
1 : 3	324	0.364
1 : 4	312	0.131

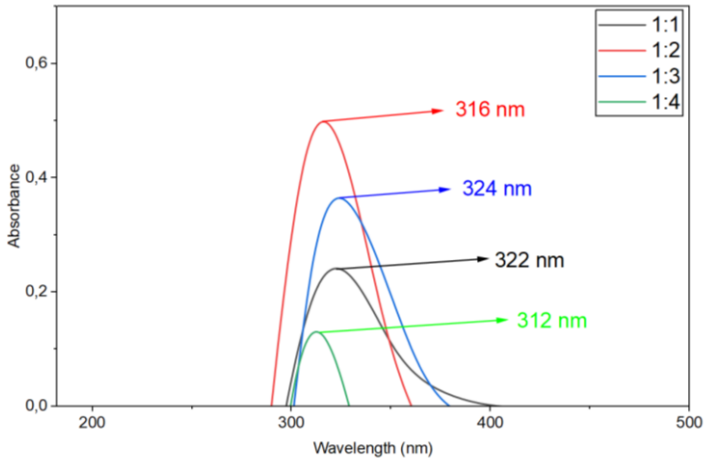


Figure 1. UV-Vis spectra of CuNPs with variation of volume

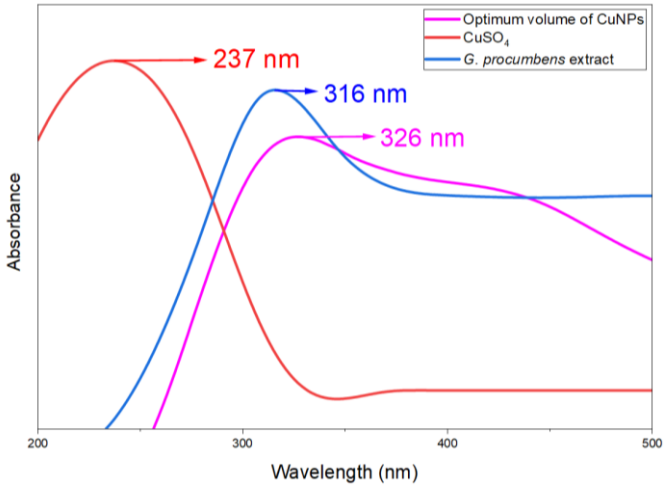


Figure 2. Comparison of UV-Vis spectra of $CuSO_4$ solution, *G. procumbens* extract, and CuNPs

However, determining the precise identity of the nanoparticles solely via UV-Vis requires caution, as copper is highly prone to oxidation. Literature indicates copper oxide nanoparticles (CuONPs) generally exhibit Surface Plasmon Resonance (SPR) peaks

between 200-300 nm [19], whereas Cu_2O at 400-500 nm [35]. The peak appearance at a wavelength of 316 nm indicates that the resulting mixture contains CuNPs, which were formed as a result of the oxidation of copper in the plant extract. Thus, this UV-Vis spectroscopy finding serves as an preliminary qualitative indicator, not as an absolute confirmation of the phase. Conversely, at higher volume ratios (1:3 and 1:4), a decrease in the capping agents from the secondary metabolites in the extract reduced the stability of the formed copper nanoparticles, thereby promoting aggregation or the formation of larger clusters. This aggregation generally reduces absorbance intensity and can shift the SPR peak due to changes in particle size and distribution [23][37].

Conversely, at higher volume ratios (1:3 and 1:4), a decrease in the capping agents from the secondary metabolites in the extract reduced the stability of the formed copper nanoparticles, thereby promoting aggregation or the formation of larger clusters. This aggregation generally reduces absorbance intensity and can shift the SPR peak due to changes in particle size and distribution [23][37].

2. Optimization pH in Synthesis CuNPs and the UV-Vis Spectroscopy Analysis

After determining the optimal conditions for CuNPs synthesis based on the volume ratio, pH optimization was conducted to determine the optimal pH. The pH optimization results are presented in Table 2 and Figure 3.

Based on these results, the optimum pH for synthesizing copper nanoparticles using *G. procumbens* leaf extract as a bioreductant was found to be pH 8, with a maximum UV–Vis absorption wavelength of 312 nm and an absorbance value of 1.284. Similar findings have also been reported in the synthesis of CuNPs using *Jasminum sambac* extract as a bioreductant, where the optimum synthesis condition was achieved at pH 8. [23].

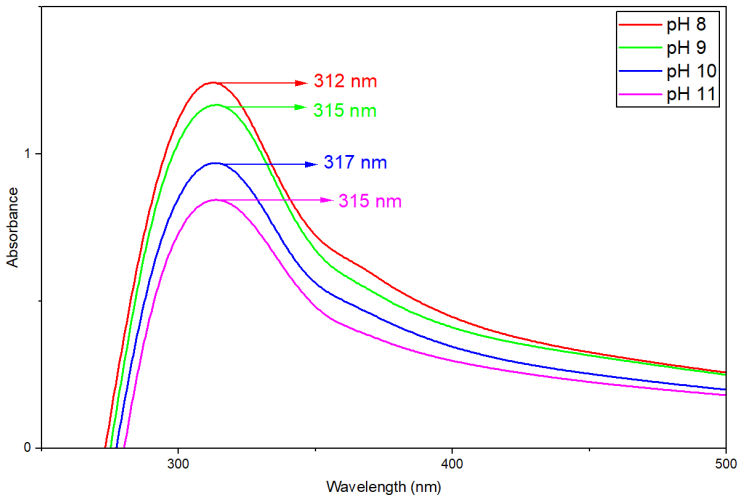


Figure 3. UV-Vis spectra from CuNPs with variation of pH

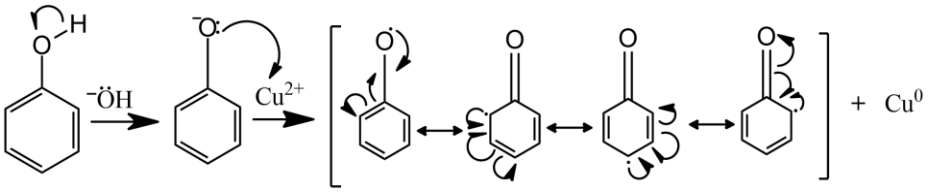


Figure 4. Mechanism of Cu^{2+} reduction by phenolic compounds in *G. procumbens* leaf extract

Table 2. The maximum absorption wavelength and absorbance of synthesized CuNPs at variation of pH

pH	λmax (nm)	Absorbance
8	312	1.284
9	315	1.165
10	317	0.971
11	315	0.844

While basic conditions generally enhance metal ion reduction by activating phenolic compounds, excess hydroxide ions can also promote side reactions. As illustrated in Figure 4, an overabundance of OH^- may cause Cu^{2+} to react and form copper(II) hydroxide ($Cu(OH)_2$), which can subsequently dehydrate into copper oxide (CuO) precipitates. In addition, pH variations significantly influence the optical properties of the resulting copper nanoparticles, leading to shifts in surface plasmon resonance (SPR) and changes in absorption intensity. Under strongly basic conditions, nucleation kinetics may slow down, potentially resulting in the formation of larger particles or aggregation [39][40].

3. Characterization of Synthesized CuNPs using Fourier Transform Infrared (FTIR)

FTIR spectroscopy was used to analyze the functional groups of the secondary metabolites. These metabolites reduce Cu^{2+} ions to Cu^0 and act as capping agents and stabilizers for the CuNPs [37]. Figure 5 a displays the FTIR spectrum of the *G. procumbens* leaf extract. It shows a broad absorption peak of the OH group from phenolic compounds at 3442.48 cm^{-1} [13], [41]. The alkyl C–H stretching vibration peak was detected at 2933.35 cm^{-1}. An aromatic C=C bond vibration peak at 1627.32 cm^{-1} supports the presence of phenolic compounds in the *G. procumbens* leaf extract. The vibration peak at 1116.29 cm^{-1} indicates C-O bonds from alcohols or ethers [13][28][36][42]. Additionally, the spectrum exhibits new absorption peaks at 457.64 and 617.14 cm^{-1}. These spectral features are consistent with Cu–O related vibrations. The appearance of these two peaks also suggest as an indication and provide a preliminary indication of copper nanoparticle [28][43].

Table 3 presents the comparison of wavenumber shifts between G. procumbens extract and CuNPs. The shift in wavenumber values and the change in absorption intensity of the OH functional group from the extract to the CuNPs indicate a significant interaction between the OH groups in the extract and Cu^{2+} ions during the reduction process. Phenolic OH groups, along with other functional groups present in flavonoids, phenolics, and tannins, are likely responsible for reducing Cu^{2+} ions to Cu^0. The reduction mechanism of Cu^{2+} ions from the $CuSO_4$

precursor by phenolic compounds is illustrated in Figure 6 [27]. The OH groups in flavonoid compounds are oxidized to quinones, while Cu^{2+} ions are reduced to CuNPs (Cu^0) [38]. Secondary metabolite compounds such as flavonoids act as capping agents that stabilize the CuNPs.

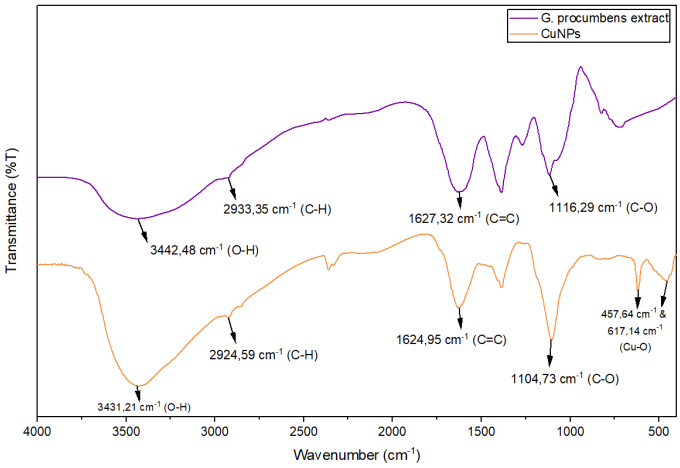


Figure 5. FTIR spectra of G. procumbens extract and CuNPs

Table 3. Comparison of wave number shift between *G. procumbens* extract and CuNPs

Functional group	*G. procumbens* extract (cm^{-1})	CuNPs (cm^{-1})	References
O-H	3442.48	3431.21	[13], [41]
C-H	2933.35	2924.59	[28]
C=C	1627.32	1624.95	[43]
C-O	1116.29	1104.73	[36]
Cu-O	-	617.14	[28]
Cu-O	-	457.64	[43]

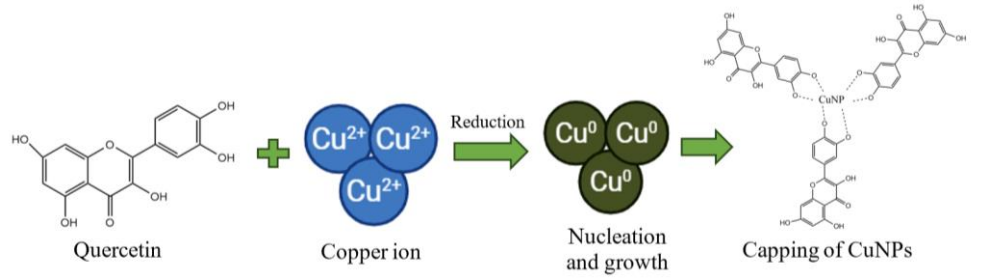


Figure 6. Hypothetic reaction between phenolic compounds and Cu^{2+} ions

4. Characterization of Synthesized CuNPs using Particle Size Analyzer (PSA)

Particle Size Analyzer (PSA) testing utilizing the Dynamic Light Scattering (DLS) principle, was performed to determine the particle size of the synthesized CuNPs [44]. DLS measures the hydrodynamic diameter of the particles in a colloidal dispersion. This measurement encompasses not only the actual metallic core but also its surrounding hydration layer and the attached secondary

metabolite capping agents, which inherently makes the measured size larger than the true core size. PSA measurement results are also influenced by the quality of the He-Ne laser and the uniformity of the colloidal dispersion. Without a uniform dispersion, the instrument detects clumps as a single large particle, which affects measurement results [45].

Based on the PSA measurements (Figure 7), the synthesized CuNPs have particle sizes ranging from 3.01 to 60.62 nm, with an average of 8.23 nm. This particle size falls within the nanoscale range (1-100 nm), confirming the formation of copper nanoparticles. The relatively small nanoparticle size (≤ 10 nm) indicates that *G. procumbens* leaf extract acts as a highly effective bioreductant for the synthesis of stable CuNPs [46].

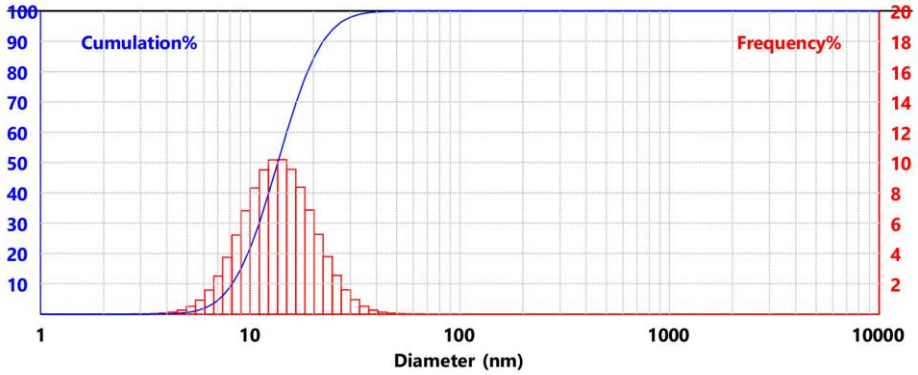


Figure 7. Characterization of CuNPs using PSA instrument

The obtained Polydispersity Index (PI) value of 0.1631 indicates that the particle size of the synthesized CuNPs falls within the homogeneous category, with a high degree of size uniformity, as it is lower than 0.50 [47]. The size of the synthesized copper nanoparticles (8.23 nm) is comparable to copper nanoparticles synthesized using *Cassia auriculata* and *Lannea discolor* leaf extracts, which obtained particle sizes of 12.43 nm [48] and 10-33 nm [13], respectively. The presence of capping agents from secondary metabolites in the extract, along with controlled reaction conditions, contributed to the formation of smaller, more stable copper nanoparticles that exhibit higher surface reactivity [12]. DLS measure the hydrodynamic diameter of particles in a dispersion, so the results can be influenced by dispersion conditions. Secondary metabolites from *G. procumbens* extract, which act as capping agents on the nanoparticle surface, can also affect the measurement. The hydration and the capping layer around the particles make the measured size larger than the actual metallic core. The broader size distribution up to 60.62 nm may indicate slight, temporary aggregation of nanoparticles. However, the low polydispersity index (PI) suggests that the system remains relatively stable and uniform.

The characterization of the synthesized copper nanoparticles in this study was limited to UV-Vis, FTIR, and PSA. Although these results are sufficient, it would be beneficial to supplement them with XRD analysis. The 2θ values of the XRD diffractogram peaks, which are specific for Cu atoms, would further support the confirmation of

the synthesized CuNPs. In addition, their crystal structure could also be determined [43].

5. Antibacterial Activity Assay Against *Escherichia coli* (ATCC 25922)

The antibacterial activity assay aimed to preliminary evaluate the effectiveness of the synthesized copper nanoparticles against the Gram-negative bacterium *Escherichia coli* (ATCC 25922). Conducted using the disk diffusion method, the results (Figure 8 and Table 4) demonstrated that the synthesized CuNPs exhibited promising antibacterial activity with a strong average inhibition zone diameter of 13.89 ± 0.45 mm. In contrast, both the pure *G. procumbens* leaf extract and the negative control showed no inhibition (0 mm) [31]. Thus, the antibacterial activity of CuNPs is stronger than that of *G. procumbens* extract.

To test the significance of the difference in antibacterial activity between CuNPs and the extract, a statistical analysis was performed. Based on the Shapiro-Wilk normality test, the antibacterial activity data for CuNPs and the extract were normally distributed ($p < 0.05$). The results of the Independent T-test showed that the antibacterial activity of CuNPs differed significantly from that of G. procumbens extract ($p < 0.01$), indicating that CuNPs significantly possess stronger antibacterial activity [33].

This clear contrast indicates that the antibacterial effect is predominantly associated with the formation of CuNPs rather than the extract itself under the tested conditions. Nanoparticle size plays a crucial role in its antibacterial properties; smaller particles increase the surface area and interaction with bacteria, thereby enhancing their antibacterial activity [49][50]. The strong antibacterial activity demonstrated by the synthesized CuNPs is supported by previous studies. For instance, CuNPs synthesized using *Ephedra alata* leaf extract exhibited strong antibacterial activity against *S. aureus* and *B. subtilis* [18], while *Morinda citrifolia* extract showed similarly strong activity against *E. coli* [19].

The toxicity mechanism of CuNPs against bacterial cells is shown in Figure 9. It begins with the release of copper ions from the nanoparticle system. These ions enter the cell, triggering the generation of reactive oxygen species (ROS). Commonly generated ROS include $O_2^{\bullet -}$, H_2O_2, and HO•. Elevated ROS levels induce oxidative stress. This damages essential molecular components, such as proteins, lipids, and DNA. As a result, bacterial cell metabolic processes are disrupted, leading to death. Furthermore, copper ions can damage DNA and interfere with ATP production by interacting with phosphate and -SH groups on proteins and DNA. This causes structural disruption, cell damage, and bacterial death. [1][12][51].

Table 4. Results of bacterial inhibition zone measurements

Sample	Diameter of Inhibition Zone (mm)				Inhibition Response of Growth
	1	2	3	Average Diameter (mm)	
G. procumbens leaf extract	-	-	-	-	-
CuNPs	14.28	13.26	14.14	13.89 ± 0.45	Strong
Positive control (ciprofloxacin)	30.72	31.10	29.32	30.38 ± 0.76	Very strong
Negative control (DDW)	-	-	-	-	-

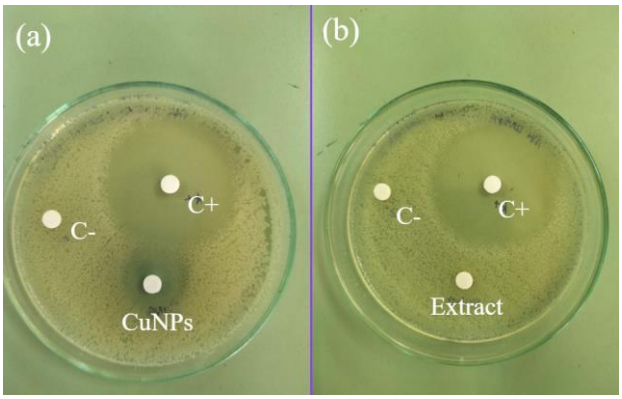


Figure 8. Antibacterial activity test of (a) CuNPs (b) *G. procumbens* leaves extract against *Escherichia coli*

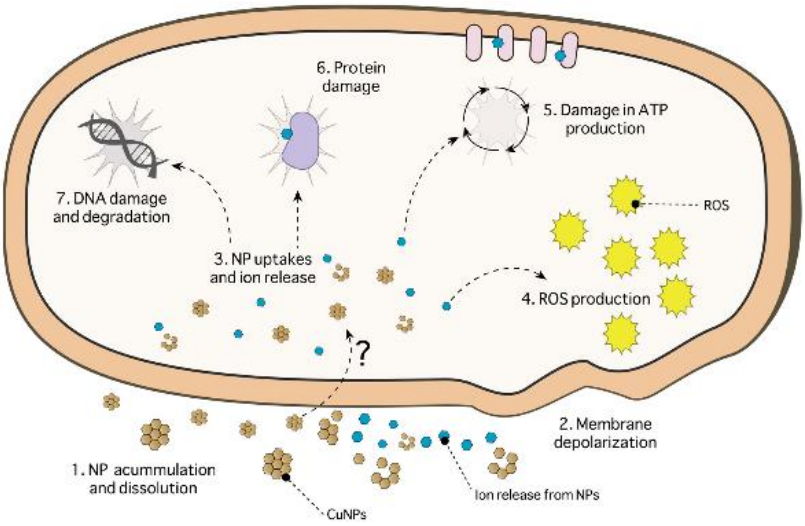


Figure 9. Antibacterial mechanism of CuNPs

The strong antibacterial activity is also facilitated by phenolic secondary metabolites from *G. procumbens* leaves, such as flavonoids, alkaloids, and tannins, which act as capping agents and stabilizers. These compounds inhibit bacterial growth by forming complexes with proteins and polysaccharides, thereby disrupting the function of various enzymes involved in intracellular enzymatic reactions [52][53][54].

These results suggest that *G. procumbens* can be used as a bioreductant for the green synthesis of CuNPs with promising antibacterial activity. These findings highlight the preliminary potential of green-synthesized CuNPs as environmentally friendly antimicrobial agents, serving as a foundational step for future explorations into their targeted applications.

Although the disk diffusion method provides a preliminary assessment of antibacterial activity, further evaluation using the dilution method is required to confirm the antibacterial potential of the synthesized CuNPs. This approach enables the determination of minimum inhibitory concentration (MIC) values and validate the true efficacy of the material [55].

CONCLUSION

This study presents the preliminary synthesis of CuNPs using G. procumbens leaf

extract, with the most favorable conditions observed at a precursor-to-extract ratio of 1:2 and a pH of 8. The synthesized CuNPs exhibited maximum UV-Vis absorption at 316 nm, while FTIR analysis indicated the presence of Cu–O functional groups, and particle size analysis revealed an average size of 8.23 nm with a Polydispersity Index (PI) of 0.1631. The CuNPs also demonstrated strong antibacterial activity against Escherichia coli, with an inhibition zone of 13.89 ± 0.45 mm. Further characterization using XRD is necessary to confirm the formation of copper nanoparticles. Meanwhile, to strengthen the potential of CuNPs as an antibacterial agent against E. coli, additional testing using the dilution method is required to determine the MIC value of the synthesized CuNPs.

REFERENCES

[1] M. L. Ermini and V. Voliani, "Antimicrobial Nano-Agents: The Copper Age," ACS Nano, vol. 15, no. 4, pp. 6008–6029, Apr. 2021, doi: 10.1021/ACSNANO.0C10756

[2] P. Szczyglewska et al., "Nanotechnology–General Aspects: A Chemical Reduction Approach to the Synthesis of Nanoparticles," Molecules, vol. 28, no. 13, Jul. 2023, doi: 10.3390/molecules28134932.

[3] B. Tawiah et al., Nanotechnology in Societal Development. Singapore: Springer, 2024. doi: 10.1007/978-981-97-6184-5_1.

[4] M. Nasrollahzadeh et al., "Plant-Mediated Green Synthesis of Nanostructures: Mechanisms, Characterization, and Applications," Interface Sci. Technol., vol. 28, pp. 199–322, Jan. 2019, doi: 10.1016/B978-0-12-813586-0.00006-7.

[5] I. Khan, et al., "Nanoparticles: Properties, applications and toxicities," Arabian J. Chemistry, vol. 12, no. 7, pp. 908–931, Nov. 2019, doi: 10.1016/J.ARABJC.2017.05.011.

[6] A. Gour and N. K. Jain, "Advances in green synthesis of nanoparticles," Artif. Cells Nanomed. Biotechnol., vol. 47, no. 1, pp. 844–851, 2019, doi: 10.1080/21691401.2019.1577878.

[7] A. Mondal and N. K. Mondal, "Efficacy of bile juice mediated Cu@MWCNT nanostructure, with respect to antibacterial and antibiofilm activity," Mater. Lett., vol. 335, p. 133828, Mar. 2023, doi: 10.1016/J.MATLET.2023.133828.

[8] F. Khan et al., "Green Nanotechnology: Plant-Mediated Nanoparticle Synthesis and Application," Nanomaterials, vol. 12, no. 4, p. 673, Feb. 2022, doi: 10.3390/nano12040673.

[9] A. Antonio-Pérez et al., "Biosynthesis of Copper Nanoparticles with Medicinal Plants Extracts: From Extraction Methods to Applications," Micromachines, vol. 14, no. 10, p. 1, Oct. 2023, doi: 10.3390/mi14101882.

[10] N. Ferdinal et al., "Identifikasi Metabolit Sekunder Dan Uji Aktivitas Antioksidan Dari Ekstrak Daun Sambung Nyawa (Gynura procumbens (Lour.) Merr).," J. Kim. Unand, vol. 12, no. 2, pp. 8–12, Nov. 2023, doi: 10.25077/JKU.12.2.8-12.2023.

[11] N. Chakraborty et al., "Green synthesis of copper/copper oxide nanoparticles and their applications: a review," Green Chem. Lett. Rev., vol. 15, no. 1, pp. 185–213, Jan. 2022, doi: 10.1080/17518253.2022.2025916;WGROUP:STRING:PUBLICATION.

[12] S. Mahmoodi et al., "Copper Nanoparticles as Antibacterial Agents," J. Mol. Pharm. Org. Process Res., vol. 6, no. 1, pp. 1–7, Jan. 2018, doi: 10.4172/2329-9053.1000140.

[13] U. Rambau et al., "Green Synthesis of Gold and Copper Nanoparticles by

Lannea discolor: Characterization and Antibacterial Activity," Inorganics., vol. 12, no. 36, p. 1, Feb. 2024, doi: 10.3390/inorganics12020036.

[14] M. A. Jobaer et al., "Phytochemical and Biological Investigation of an Indigenous Plant of Bangladesh, Gynura procumbens (Lour.) Merr.: Drug Discovery from Nature," Molecules, vol. 28, no. 10, p. 4186, May 2023, doi: 10.3390/molecules28104186.

[15] M. Chandradevan et al., "UHPLC-ESI-Orbitrap-MS Analysis of Biologically Active Extracts from Gynura procumbens (Lour.) Merr. and Cleome gynandra L. Leaves," Evid. Based Complement. Alternat. Med., vol. 2020, no. 1, p. 3238561, Jan. 2020, doi: 10.1155/2020/3238561.

[16] M. I. Din et al., "Green Adeptness in the Synthesis and Stabilization of Copper Nanoparticles: Catalytic, Antibacterial, Cytotoxicity, and Antioxidant Activities," Nanoscale Res. Lett., vol. 12, no. 1, pp. 1–15, Dec. 2017, doi: 10.1186/S11671-017-2399-8.

[17] T. M. Le Quyen et al., "Chemical constituents and the inhibition of α-glucosidase of Gynura procumbens (Lour.) Merr.," Sci. Technol. Dev. J., vol. 22, no. 4, pp. 391–391, 2019, doi: 10.32508/stdj.v22i4.1725.

[18] A. Atri et al., "Green synthesis of copper oxide nanoparticles using Ephedra Alata plant extract and a study of their antifungal, antibacterial activity and photocatalytic performance under sunlight," Heliyon, vol. 9, no. 1, pp. 2405–8440, 2023, doi: 10.1016/j.heliyon.2023.e13484.

[19] M. Priya et al., "Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from Morinda citrifolia leaf extract," Sci. Rep., vol. 13, p. 18838, Nov. 2023, doi: 10.1038/s41598-023-46002-5.

[20] H. R. Naika et al., "Green synthesis of CuO nanoparticles using Gloriosa superba L. extract and their antibacterial activity," J. Taibah Univ. Sci., vol. 9, no. 1, pp. 7–12, 2015, doi: 10.1016/J.JTUSCI.2014.04.006.

[21] A. Ullah et al. "Bioinspired green synthesis of copper, nickel, and hybrid nanoparticles using Myristica Fragrans seeds: Biomedical applications and beyond," Mater. Res. Express, vol. 11, no. 8, p. 085401, Aug. 2024, doi: 10.1088/2053-1591/AD68CF.

[22] S. Sutoyo et al., "Synthesis and Characterization of Copper Nanoparticles Using Carica Dieng (Carica pubescens) Seed Extract As A Bioreducer And Testing Their Antibacterial Activity," Rasayan J. Chemistry, vol. 18, no. 2, pp. 819–824, Apr. 2025, doi: 10.31788/RJC.2025.1829159.

[23] S. Nouren et al., "Green synthesis of CuO nanoparticles using Jasmin sambac extract: Conditions optimization and photocatalytic degradation of Methylene Blue dye," J. King Saud Univ. Sci., vol. 36, no. 3, p. 103089, Mar. 2024, doi: 10.1016/j.jksus.2024.103089.

[24] M. Sulistiorini et al., "Synthesis and Characterization of Silver Nanoparticles Using Bioreductant Andong Leaf Extract (Cordyline fruticosa (L) A. Chev.)," J. Kim. Sains Apl., vol. 27, no. 5, pp. 243–249, May 2024, doi: 10.14710/jksa.27.5.243-249.

[25] WHO, "Diarrhoeal disease," 2026. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease.

[26] M. M. Nadzir et al., "Green synthesis of silver nanoparticle using Gynura procumbens aqueous extracts," in AIP Conf. Proc., American Institute of Physics Inc., Jul. 2019. doi: 10.1063/1.5117140.

[27] C. A. Haruna et al., "Green Synthesis of Copper Nanoparticles Using Red Dragon Fruit (Hylocereus polyrhizus) Extract and Its Antibacterial Activity

for Liquid Disinfectant," J. Kim. Sains Apl., vol. 25, no. 10, pp. 352–361, 2022. doi: 10.14710/jksa.25.10.352-361

[28] R. W. H. Putri and S. Sutoyo, "The Potency of Noni Leaves Extract (Morinda citrifolia L.) as a Bioreductor in the Synthesis of Copper Nanoparticles and Its Effectiveness as an Antibacterial against Streptococcus pyogenes," J. Kim. Sains Apl., vol. 28, no. 3, pp. 138–145, Apr. 2025, doi: 10.14710/jksa.28.3.138-145.

[29] S. C. R. R. Rajamanikkam et al., "Green Synthesis of Copper and Copper Oxide Nanoparticles From Brown Algae Turbinaria Species' Aqueous Extract and Its Antibacterial Properties," Cureus, vol. 16, p. e57366, Apr. 2024, doi: 10.7759/cureus.57366.

[30] H. A. Muiz et al., "Uji Aktivitas Aantibakteri Ekstrak Daun Patikan Kebo (Euphorbia hirta L) Terhadap Staphylococcus aureus Dengan Metode Difusi Cakram," J. Anal. Farm., vol. 6, no. 2, pp. 84–89, Jan. 2021, doi: 10.33024/JAF.V6I2.5942.

[31] Y. S. Senge et al., "Phytochemical Screening and Antibacterial Activity Of Ethanolic Extracts from Delonix regia Against Laboratory Strains Of Diarrheal Bacteria," JKPK (Jurnal Kimia dan Pendidikan Kimia), vol. 9, no. 1, 2024, doi: 10.20961/jkpk.v9i1.85374.

[32] Emelda et al., "Aktivitas Inhibisi Ekstrak Etanolik Ulva lactuca terhadap Bakteri Staphylococcus aureus," Pharmaceutical Journal of Indonesia, vol. 7, no. 1, pp. 43–48, 2021, doi: https://doi.org/10.21776/ub.pji.2021.007.01.7.

[33] S. Abubakar, I. Musa, S. Maigida, A. Halliru, and E. Menyechi, Beginner's Guide to IBM SPSS Statistics: Data Entry, Cleaning and Variable Transformation, 2026. DOI: 10.14710/jksa.25.10.352-361.

[34] M. J. Sultana et al., "Biosensing and anti-inflammatory effects of silver, copper and iron nanoparticles from the leaf extract of Catharanthus roseus," Beni. Suef. Univ. J. Basic Appl. Sci., vol. 12, no. 26, pp. 1–7, 2023, doi: 10.1186/s43088-023-00358-9.

[35] A. R. Carrasco-Hernández et al., "Evolution of Structural and Optical Properties of Cuprous Oxide Particles for Visible Light Absorption," J. Nanomater., vol. 2022, no. 7964428, pp. 1–15, 2022, doi: 10.1155/2022/7964428.

[36] F. Arodiya, et al., "Green Synthesis of Copper Nanoparticles with Adhatoda Vasica: Antibacterial and Antioxidant Study," J. Chem. Environ., vol. 4, no. 2, pp. 1–10, Aug. 2025, doi: 10.56946/JCE.V4I2.660.

[37] A. Muhammad et al., "Green synthesis of copper nanoparticles using Musa acuminata aqueous extract and their antibacterial activity," Asian J. Trop. Biotechnol., vol. 20, no. 1, pp. 10–16, Feb. 2023, doi: 10.13057/BIOTEK/C200102.

[38] S. Amaliyah et al., "Green synthesis and characterization of copper nanoparticles using Piper retrofractum Vahl extract as bioreductor and capping agent," Heliyon, vol. 6, no. 8, p. e04636, Aug. 2020, doi: 10.1016/j.heliyon.2020.e04636.

[39] S. L. Valan et al., "Sustainable Synthesis of Copper Oxide Nanoparticles Using Aquilaria Malaccensis (Agarwood) Leaf Extract as Reducing Agent," Int. J. Technol., vol. 13, no. 5, pp. 1115–1125, 2022, doi: 10.14716/ijtech.v13i5.5845.

[40] R. Amjad et al., "Green synthesis and characterization of copper nanoparticles using fortunella margarita leaves," Polymers, vol. 13, no. 24, p. 4364, Dec. 2021, doi: 10.3390/polym13244364.

[41] D. M. Nzilu et al., "Green synthesis of copper oxide nanoparticles and its

efficiency in degradation of rifampicin antibiotic," Sci. Rep., vol. 13, p. 14030, 2023, doi: 10.1038/s41598-023-41119-z.

[42] K. Okaiyeto et al. "Plant-Based Synthesis of Silver Nanoparticles Using Aqueous Leaf Extract of Salvia officinalis: Characterization and its Antiplasmodial Activity," J. Clust. Sci., vol. 32, pp. 101–109, 2021, doi: 10.1007/s10876-020-01766-y.

[43] S. K. Chandraker et al., "Green synthesis of copper nanoparticles using leaf extract of Ageratum houstonianum Mill. and study of their photocatalytic and antibacterial activities," Nano Express, vol. 1, pp. 010033–12, 2020, doi: 10.1088/2632-959X/ab8e99.

[44] A. Amin and S. Tengker, "Sintesis dan Karakterisasi Nano ZnO Menggunakan Bioreduktor Ekstrak Daun Kopasanda (Chromolaena odorata L.)," Fullerene J. Chem., vol. 7, no. 1, pp. 47–51, Mar. 2023, doi: 10.37033/FJC.V7I1.511.

[45] S. Lukač et al., "Synthesis, Characterisation, and Biological Assessment of Chromium Oxide Nanoparticles Coated with Chia Seed Mucilage Extract," Pharmaceutics, vol. 18, no. 1, p. 49, Dec. 2025, doi: 10.3390/pharmaceutics18010049.

[46] P. K. Dikshit et al., "Green Synthesis of Metallic Nanoparticles: Applications and Limitations," Catalysts, vol. 11, no. 8, p. 902, Jul. 2021, doi: 10.3390/catal11080902.

[47] K. T. A. Dewi et al., "Karakter Fisik dan Aktivitas Antibakteri Nanopartikel Perak Hasil Green Synthesis Menggunakan Ekstrak Air Daun Sendok (Plantago major L.)," Pharm. Sci. Res., vol. 6, no. 2, pp. 69–81, 2019, doi: https://doi.org/10.7454/psr.v6i2.4220.

[48] C. C. Manasa et al., "Green Synthesis of Copper Nanoparticles Using Leaves and Flowers Extract of Cassia auriculata L. and Study of It's Antimicrobial Activity.," Int. J. Res. Appl. Sci. Eng. Technol., vol. 11, no. 12, pp. 468–482, Dec. 2023, doi: 10.22214/IJRASET.2023.57361.

[49] L. Wang et al., "The antimicrobial activity of nanoparticles: present situation and prospects for the future," Int. J. Nanomedicine, pp. 1227–1249, 2017, doi: 10.2147/IJN.S121956.

[50] M. Ozdal and S. Gurkok, "Recent advances in nanoparticles as antibacterial agent," ADMET DMPK, vol. 10, no. 2, p. 115, 2022, doi: 10.5599/ADMET.1172.

[51] I. A. Ivanova et al., "Copper and Copper Nanoparticles Applications and Their Role against Infections: A Minireview," Processes, Vol. 12, Page 352, vol. 12, no. 2, p. 352, Feb. 2024, doi: 10.3390/PR12020352.

[52] Nurhasanah and E. S. Gultom, "Uji Aktivitas Antibakteri Ekstrak Metanol Daun Kirinyuh (Chromolaena odorata) Terhadap Bakteri MDR (Multi Drug Resistant) Dengan Metode KLT Bioautografi," J. Biosains, vol. 6, no. 2, pp. 45–52, Aug. 2020, doi: 10.24114/jbio.v6i2.16600.

[53] R. Al Kausar et al., "Skrinning Fitokima Dan Uji Daya Hambat Ekstrak Daun Kenikir (Cosmos caudatus Kunth) Terhadap Bakteri Staphylococcusaureus Dengan Metode Difusi Cakram," J. Anal. Farm., vol. 8, no. 1, pp. 77–89, Apr. 2023, doi: https://doi.org/10.33024/jaf.v8i1.9913

[54] Y. Mulyani et al., "Review: Kajian Aktivitas Antibakteri dan manifestasinya dari Tanaman Sambung Nyawa (Gynura procumbens)," J. Mandala Pharmacon Indonesia, vol. 7, no. 2, pp. 123–142, Dec. 2021, doi: https://doi.org/10.35311/jmpi.v7i2.81

[55] T. J. Hossain, "Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations," Eur. J. Microbiol. Immunol., vol. 14, no. 2, p. 97, Apr. 2024, doi: 10.1556/1886.2024.00035.