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Mechanical Grinding-Derived Turmeric Rhizome Nanoparticles (*Curcuma longa* L.): Characterization and Comparative Antibacterial Efficacy

Bina Melvia Girsang¹, Hakim Bangun², Yuandani³, Muhammad Rusda⁴, Tengku Ismanelly Hanum⁵

¹ Doctor of Pharmaceutical Sciences Programme, Faculty of Pharmacy, Universitas Sumatera Utara, Medan Indonesia.

² Department of Pharmaceutical and Technology, Faculty of Pharmacy and Health Sciences, Universitas Sari Mutiara Indonesia

³ Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

⁴ Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia

⁵ Department of Pharmaceutical and Technology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia

ABSTRACT: Antimicrobial resistance, especially to *Staphylococcus aureus* (*S.aureus*), is a serious global health problem because of the bacteria's ability to resist treatment for various infections and the high level of bacterial resistance. This study investigated the physicochemical characterization and antibacterial activity of turmeric (*Curcuma longa* L) rhizome powder and its derived nanoparticles prepared via mechanical milling. The nanoparticles were synthesized through top-down mechanical milling with varying grinding durations (30, 60, 90, and 120 min). Comprehensive characterization was performed using Scanning Electron Microscopy with Energy Dispersive X-ray analysis (SEM-EDX), X-ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR), and Differential Scanning Calorimetry (DSC). Particle size distribution and active compound content (curcuminoid) were quantitatively determined. Antibacterial efficacy was evaluated against *S.aureus*. Based on particle size analysis (PSA) and particle morphology (SEM), the optimal grinding duration was 60 min, producing fine, uniform, agglomeration-free nanoparticles (117.16 nm). The EDX results showed the best elemental composition at 30 minutes. DSC analysis showed the best thermal stability of the nanoparticles ground for 30 min, while FTIR showed the best-preserved curcuminoid structure at 60 min. Grinding duration affected ash content, with the optimum result (6.19%) also found at 60 min. Antibacterial activity showed the lowest % MIC (0.905%) at 24 hours with a grinding duration of 30 min, and there was a significant effect ($p < 0.05$) between the grinding duration and the inhibition zone of *S. aureus*. This study provides a scientifically rigorous, mechanistically grounded foundation. The formulations may offer improved with significantly enhanced biopharmaceutical performance compared with conventional turmeric preparations.

1. INTRODUCTION

Antimicrobial resistance, particularly in *Staphylococcus aureus* (*S.aureus*), has become a significant global health threat due to the ability of pathogens to resist a wide range of drugs and the high mortality rates associated with severe infections [1]. The lack of new antibiotic classes emerging in recent decades has exacerbated this problem, thus highlighting the need for innovative treatment strategies [1].

Turmeric (*Curcuma longa*) is recognized for its therapeutic benefits, primarily attributed to curcumin [2]. Recent studies have emphasized the antibacterial activity of turmeric, largely due to curcumin and other rhizome phytochemicals [3]. *S. aureus*, a Gram-positive bacterium responsible for various infections, presents a growing public health risk owing to antibiotic resistance, particularly in MRSA strains [4,5]. Turmeric contains several bioactive compounds, with curcumin being the most prominent [6]. Curcumin has been shown to have anti-inflammatory, antioxidant, and antibacterial effects. It can also disrupt bacterial membranes, inhibit biofilm formation, and affect signaling pathways [7].

Other phytochemicals in turmeric, such as demethoxycurcumin, bisdemethoxycurcumin, and curcumin also enhance its antibacterial activity [8]. However, curcumin's clinical use is limited by poor bioavailability, rapid metabolism, and low water solubility [3,9]. Nanotechnology is a promising strategy to overcome these limitations, as converting turmeric into nanoparticles can improve bioavailability and strengthen its antibacterial effects by increasing surface area and interaction with bacterial membranes [10,11].

Although turmeric nanoparticles show promise, their antibacterial mechanisms and the influence of production methods, such as milling, are not yet fully understood [12,13,11,14]. This study focused on how milling variations affect the antibacterial efficacy of turmeric nanoparticles against *S. aureus*. By combining advanced nanoparticle characterization with microbiological testing, this research aims to clarify how production methods relate to antibacterial activity. The integration of complementary analytical test parameters will provide an unprecedented holistic mechanistic understanding of nano-transformation processes. The findings could significantly enhance the scientific and applied rigor of this research, providing a foundation for understanding raw-material processing using pharmaceutical nanotechnology.

Through a systematic analysis of turmeric concentration and milling size, this study seeks to identify the optimal conditions to maximize antibacterial activity. Advancements in green nanotechnology continue to address safety concerns associated with drug production [15-20]. These findings are expected to deepen understanding of the antibacterial mechanisms of turmeric and support its use in pharmaceutical applications [21].

2. MATERIALS AND METHODS

2.1 Study Material

The reagents included distilled water dimethyl sulfoxide (DMSO, Merck, Germany), ethanol (96%, Merck, Germany), povidone-iodine (10%, generic pharmaceutical grade), and Mueller-Hinton agar (Himedia). The test organism, *S. aureus* ATCC 29213, was sourced from Agavi Laboratory, Bandung, Indonesia.

2.2 Sample Collection

Turmeric Rhizome Powder and Nanoparticle Powder Preparations

Fresh, pest-free turmeric rhizomes were selected, washed under running water until clean, and then sanitized with 70% ethanol for 1 minute. After draining, the rhizomes were weighed, sliced to 0.1 cm thickness, and arranged on a stainless drying mat before drying at 40°C. Dryness was checked periodically over two days to ensure they were brittle and golden orange. The dried slices were blended into a powder, sieved through a 100 mesh, and then subjected to mechanical grinding for 30, 60, 90, and 120 minutes as described in Figure 1.



Fig.1 Fabrication of Powder and Nanoparticles of Turmeric Rhizome Powder Preparations

2.3 Sample Collection

The prepared turmeric samples (STRP, TRP, and TRPNG) were obtained through a series of processing steps, including cleaning, ethanol washing, drying at 40 °C, slicing, grinding, and sieving to 100 mesh size. The dried turmeric rhizomes were then converted into powder and subjected to mechanical grinding for 30, 60, 90, and 120 minutes to produce nanoparticle-sized samples (TRPNG). The resulting samples were then used for further characterization analyses as presented in the following sections.

2.4 Analytical Methods

2.4.1. Physical characteristics

Turmeric powder and nanoparticle samples ground for 30, 60, 90, and 120 minutes were examined for water content, total ash, and acid-insoluble ash.

2.4.2. Curcuminoid Content Testing

Curcumin content was analyzed at Green Lab, Bandung, using high-performance liquid chromatography (HPLC, Shimadzu Corporation, Japan) with curcumin standards. Levels of bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin (C) were measured. For the separation, 25 mg of each sample (powder or nanoparticle ground for 30, 60, 90, or 120 minutes) was sonicated in 50 mL of reagent alcohol, then further diluted 1:25. Curcumin standard solutions at 10 µg/mL were prepared in methanol, with aliquots adjusted for standard dilutions. Solvent and wavelength optimization was performed, with maximum absorption at 425 nm. Method validation included experimental determination of the limit of detection (LOD) and limit of quantification (LOQ) following established guidelines [22].

2.4.3 Scanning Electron Microscopy) (SEM) and Energy Dispersive X-ray (EDX)

SEM characterization was performed at the FMIPA Physics Laboratory, Syah Kuala University, using a Thermo Scientific Prisma SEM (Version 16.0) equipped with an Oxford Instruments EDS detector. Samples were mounted on carbon tape and imaged at 1000×, 5000×, and 10,000× magnifications.

2.4.4. Particle Size Analyzer (PSA)

Turmeric rhizome powder nanoparticles were produced using a top-down method at the Integrated Research Laboratory, Universitas Sumatera Utara. Powder and grinding balls were placed in a pre-washed HEM-E3D jar and milled with a High Energy Ball Mill (Emax Retsch GmbH) at 2,000 min⁻¹. Particle size analysis was then conducted with a Fritsch Particle Size Analyzer Nanotech 22 at the Nanomedicine Center of Innovation Laboratory, Universitas Sumatera Utara.

X-Ray Diffraction (XRD) Examination

X-ray techniques are effective for evaluating turmeric quality and composition, with optimal radiography parameters previously set at 22–25 kV and 3–5 mA [23]. In this study, the XRD-7000 instrument, operated at 40 kV and 30 mA was used for analysis at FMIPA, Syah Kuala University.

2.4.5. Differential Scanning Calorimetry (DSC)

The DSC-600-II Thermal Analyzer was used to assess turmeric, particularly curcumin, via differential scanning calorimetry (DSC) determining melting point, thermal stability, purity, and crystalline-to-amorphous forms transitions [24].

2.4.6. Fourier Transform Infrared Spectroscopy FTIR

FTIR analysis was conducted to identify the functional groups present in the samples. The spectra were recorded using an FTIR spectrometer (Shimadzu Corporation, Japan) over a wavenumber range of 4000–400 cm^{-1} . Prior to the analysis, each sample (standard turmeric rhizome powder (STRP), turmeric rhizome powder (TRP), and turmeric rhizome powder nanoparticle grinding (TRPNG) at 30, 60, 90, and 120 minutes) was dried and finely homogenized. The samples were then prepared using the potassium bromide (KBr) pellet method by thoroughly mixing the sample with powder and compressing it into a transparent pellet under high pressure, then analyzed to obtain FTIR spectra, which were interpreted in the results section [25].

2.4.7. Antibacterial Activity

1 gram of turmeric powder or nanoparticle powder (TRPNG, 30–120 minutes) was weighed and dissolved in DMSO to a total volume of 10 mL, thus yielding a concentration of 100 mg/mL. Serial dilutions were then prepared at concentrations of 1, 2, 3, 4, 5, 10, 20, 40, 60, 80, and 100 mg/mL.

The well diffusion method was performed in four replicates. An inoculum (0.1 mL) was mixed with 15 mL of Mueller-Hinton agar in sterile Petri dishes. After solidification, wells were loaded with each sample concentration, DMSO as a negative control, and 10% povidone-iodine as a positive control. Plates were incubated at 37°C for 24 hours, and inhibition zone diameters were measured with a vernier caliper [26].

The minimum inhibitory concentration (MIC) was determined on the first day (24 hours). Previous studies reported significant antimicrobial activity of curcumin against *S. aureus* after 18–24 hours. MIC values were calculated from the average square diameter (X^2) of inhibition zones and the natural logarithm of sample concentrations on day 1, with correlation coefficients and equations obtained for each sample type.

The minimum inhibitory concentration (MIC) value is determined based on the following equation:

$$\ln(\text{MIC}) = \ln\left[\frac{X^2}{4Dt}\right] \ln(c) \quad (1)$$

MIC: Minimum Inhibitory Concentration

x: Inhibitory Diameter

D: Diffusion Coefficient

t: Diffusion Time

Equation (1) is then derived as:

$$\ln(c) = \ln(\text{MIC}) \frac{X^2}{4Dt} \quad (2)$$

[27,28].

The MIC value is obtained by plotting $\ln(c)$ vs X^2 , and taking the antilogarithm of the intercept.

The long-term effect of incubation on MIC values was tested with an incubation period of 7 days and monitoring 4 times after 24 hours:

1. Turmeric Rhizome Powder (TRP)
2. Turmeric Rhizome Powder Nanoparticles Grinding (TRPNG) 30, 60, 90, 120 minutes

2.4.8. Phytochemical Screening

Phytochemical screening was conducted to identify the secondary metabolites in turmeric rhizome powder by observing color changes using specific reagents and solvents.

2.5 Statistical Analysis

Statistical tests were carried out on the antibacterial activity, and statistical analysis of the correlation coefficients of the regression equations was performed for the minimum bacterial inhibition zones within the first 24 hours for nanoparticle powders with grinding durations of 30, 60, 90 and 120 minutes. Furthermore, the differences in the effect of turmeric rhizome grinding duration at 24 hours on the *S. aureus* bacterial inhibition zone were analysed using *One-way ANOVA*.

3. RESULTS AND DISCUSSION

3.1 Preliminary Analysis

3.1.1. Physical characteristics

Physical properties indicate the quality of the powder, as a high moisture content can promote microbial growth and decrease the stability of active compounds [29]. The physical characteristics of turmeric rhizome (*Curcuma longa*, L) preparations are presented in Table 1..

Table 1. Physicochemical characteristics of the Turmeric Rhizome Powder and Nanoparticle Grinding at 30,60,90,120 Minutes

No	Parameter	TRP 0 Minute	TRPNG 30 Minutes	TRPNG 60 Minutes	TRPNG 90 Minutes	TRPNG 120 Minutes
1.	Water Content (Not > 10%)	8.62	7.427	8.65	8.65	12.4
2.	Water Soluble Extract Content (Not < 11.5%)	19.45	20.45	20.45	20.45	20.45
3.	Soluble Extract Content in Ethanol (Not less than 11.4%)	21.33	33.93	32.2	21	25.96
4.	Ash content (not > 8.2%)	7.23	7.648	6.19	6.73	6.8
5.	Acid Insoluble Ash Content (Not > 0.9%)	1.67	0.897	0.89	1.05	1.04

The physicochemical properties of TRP and TRPNG at different grinding durations (30, 60, 90, and 120 minutes) are presented in Table 1. The initial turmeric rhizome powder (TRP, 0 minute) showed a water content of 8.62%, which is below the maximum limit of $\leq 10\%$ as specified by the Indonesian Pharmacopoeia (K. R. Kumar & Reddy, 2022). After 30 minutes of grinding, the

water content decreased to 7.427%, indicating effective removal of surface moisture. However, further grinding at 60 and 90 minutes resulted in an increase to 8.65%, likely due to heat generation and friction during mechanical processing. At 120 minutes, the water content exceeded the acceptable limit, reaching 12.4%. This trend was consistent with Sapariya (2015), who reported that grinding processes significantly influence moisture retention in turmeric powder [30].

The water-soluble extract content remained relatively stable across samples. TRP recorded a value of 19.45%, while all TRPNG samples (30–120 minutes) consistently showed higher values of 20.45%, indicating improved solubility and extractability after mechanical size reduction. In contrast, the ethanol-soluble extract content exhibited a more variable pattern. A marked increase was observed at 30 minutes (33.93%) compared to TRP (21.33%), followed by a decrease at 60 minutes (32.2%) and 90 minutes (21%), before increasing again at 120 minutes (25.96%). These fluctuations therefore suggest that grinding time affects the release and availability of ethanol-soluble phytoconstituents from the turmeric matrix.

For the ash-related parameters, all of the samples remained within the acceptable limit (<8.2%). The ash content of TRP was 7.23%, while TRPNG samples ranged from 6.19% to 7.648%, indicating an overall reduction after grinding. Similarly, acid-insoluble ash content decreased from 1.67% (TRP) to 0.89–1.05% in TRPNG samples, suggesting improved purity and reduced inorganic impurities following nanoparticle processing. A decreasing trend in the ash content was observed up to 60 minutes of grinding, reaching the lowest value (6.19%), which may be associated with improved homogenization and dispersion of inorganic constituents due to increased surface area.

Beyond 60 minutes, the ash content increased slightly at 90 and 120 minutes (6.73% and 6.8%, respectively). This may indicate that prolonged grinding disrupts plant cell structure, potentially releasing bound mineral components such as potassium, magnesium, and silica. In addition, over-grinding may lead to partial particle re-agglomeration, which can affect the distribution of inorganic residues. Thus, grinding duration plays a critical role in determining ash characteristics, with 60 minutes appearing as the most effective condition for minimizing ash content. These findings are consistent with Óze and Makó (2023), who reported that mechanochemical processing enhances material dispersion, while excessive grinding may induce re-agglomeration and alter the physicochemical properties [31]. Similarly, Jabbar (2025), highlighted that variations in grinding time influence ash behavior and active compound distribution through improved homogeneity and mild thermal effects [11].

Phytochemical screening of turmeric rhizome and turmeric rhizome nanoparticle crude extract was carried out to identify the presence of active secondary metabolites using standard procedures to detect terpenoids, steroids, flavonoids, phenolic compounds, and tannins [32].

Alkaloids, flavonoids, terpenoids, and tannins were consistently detected and they remained stable across different grinding durations. In contrast, steroids and saponins appeared more susceptible to extended grinding, likely due to thermal degradation or structural changes.

3.1.2. Curcuminoid Content Testing

The table below presents the levels of the main bioactive compounds in turmeric rhizome (*Curcuma longa* L), BDMC (Bisdemethoxycurcumin), DMC (Demethoxycurcumin), and Curcumin (C) measured in the turmeric powder and nanoparticle forms after 30, 60, 90, and 120 minutes of grinding. The values are shown as mean (X) and standard deviation (SD), reflecting measurement accuracy and data variability for each sample.

Table 2. Curcuminoid content of Turmeric Rhizome Powder and Turmeric Rhizome Powder Nanoparticle Grinding at various grinding times

No	The active ingredient in turmeric	TRP	TRPNG-30	TRPNG-60	TRPNG-90	TRPNG-120	<i>P-value</i>
		X±SD	X±SD	X±SD	X±SD	X±SD	
1	BDMC (%w/w)	0.281 ± 0.0078	0.313 ± 0.015	0.316 ± 0.015	0.001 ± 0.389	0.373 ± 0.005	<0.001*
2	DMC (%w/w)	0.406 ± 0.0115	0.389 ± 0.0011	0.446 ± 0.0057	0.490 ± 0.001	0.473 ± 0.005	0.310*
3	Curcumin (C) (%) w/w)	4.777 ± 0.0208	4.852 ± 0.0590	4.928 ± 0.0243	4.607 ± 0.006	4.603 ± 0.005	<0.001*

One-way ANOVA; TRP (Turmeric Rhizome Powder); TRPNG (Turmeric Rhizome Powder Nanoparticle Grinding (30, 60, 90, 120 minutes); BDMC (Bisdemethoxycurcumin); DMC (Demethoxycurcumin); C (Curcumin).

The BDMC content rose from 0.281% in regular powder to 0.313% and 0.316% in nanoparticles after 30 and 60 minutes of grinding, suggesting better compound release with a reduced particle size. However, grinding for 90 minutes led to BDMC degradation, likely from excess heat and oxidation [33], consistent with findings by Yun et al [34,35], consistent with findings reported by Naksuriya et al. (2016) and Yun et al. (2025), who reported curcuminoid sensitivity to mechanical and thermal stress. DMC content decreased slightly at 30 minutes (0.389% vs. 0.406% in regular powder), but then increased at 60, 90, and 120 minutes (0.446%, 0.490%, and 0.473%), likely due to enhanced release from greater surface area [36]. An overview of active compound levels is shown in Fig. 2.

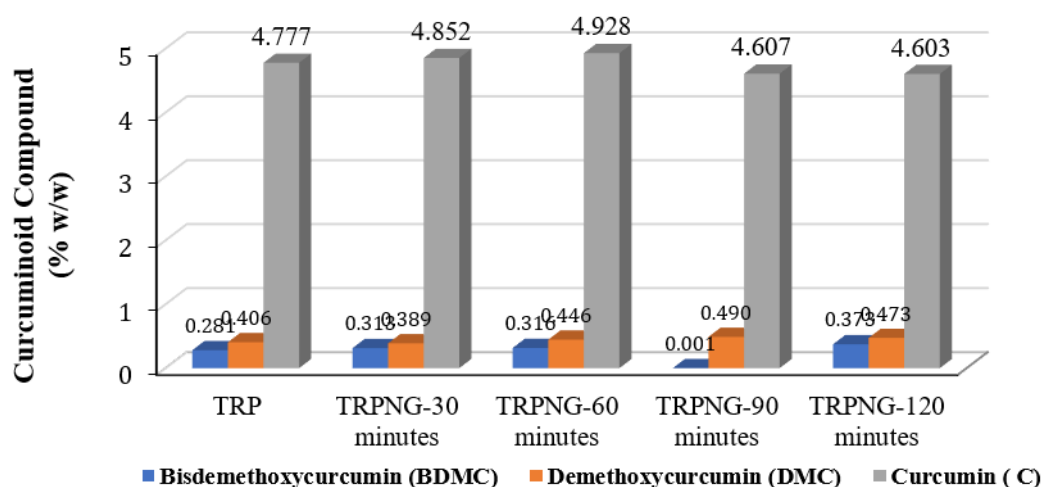


Fig. 2. Curcumin Active Ingredients: Bisdemethoxycurcumin (BDMC), Desmethoxycurcumin (DMC), Curcumin (C) in TRP (Turmeric Rhizome Powder) (TRP) and TRPNG (Turmeric Rhizome Powder Nanoparticle Grinding (30, 60, 90, 120 minutes)).

Curcuminoid compound levels were summarized using HPLC. Curcumin concentration peaked at 60 minutes (4.928 mg/g) but declined with further grinding, suggesting that prolonged grinding may damage curcumin's chemical structure, likely due to thermal or oxidative degradation (Esatbeyoglu et al., 2015). The curcumin content in turmeric powder increased from $4.77 \pm 0.02\%$ at 0 minutes to a peak of $4.922 \pm 0.024\%$ at 60 minutes, then dropped to $4.60 \pm 0.006\%$ at 90 minutes and $4.603 \pm 0.005\%$ at 120 minutes, reflecting the impact of grinding duration on curcumin stability.

The rise in the curcumin content from 0 to 60 minutes is attributed to reduced particle size, which increases surface area for extraction. Smaller particles enhance curcumin release, leading to higher measured concentrations. This is consistent with Lu (2020), who found that grinding improves bioavailability of compounds like curcumin by increasing surface area and breaking down cell structures [37].

The decline in curcumin levels after 60 minutes is likely caused by thermal or oxidative degradation from prolonged grinding. Excessive mechanical energy can generate enough heat to damage or alter curcumin's structure, as it is sensitive to high temperatures, light, and pH [38]. Additionally, extended grinding may lead to particle re-agglomeration, reducing the availability of curcumin [39].

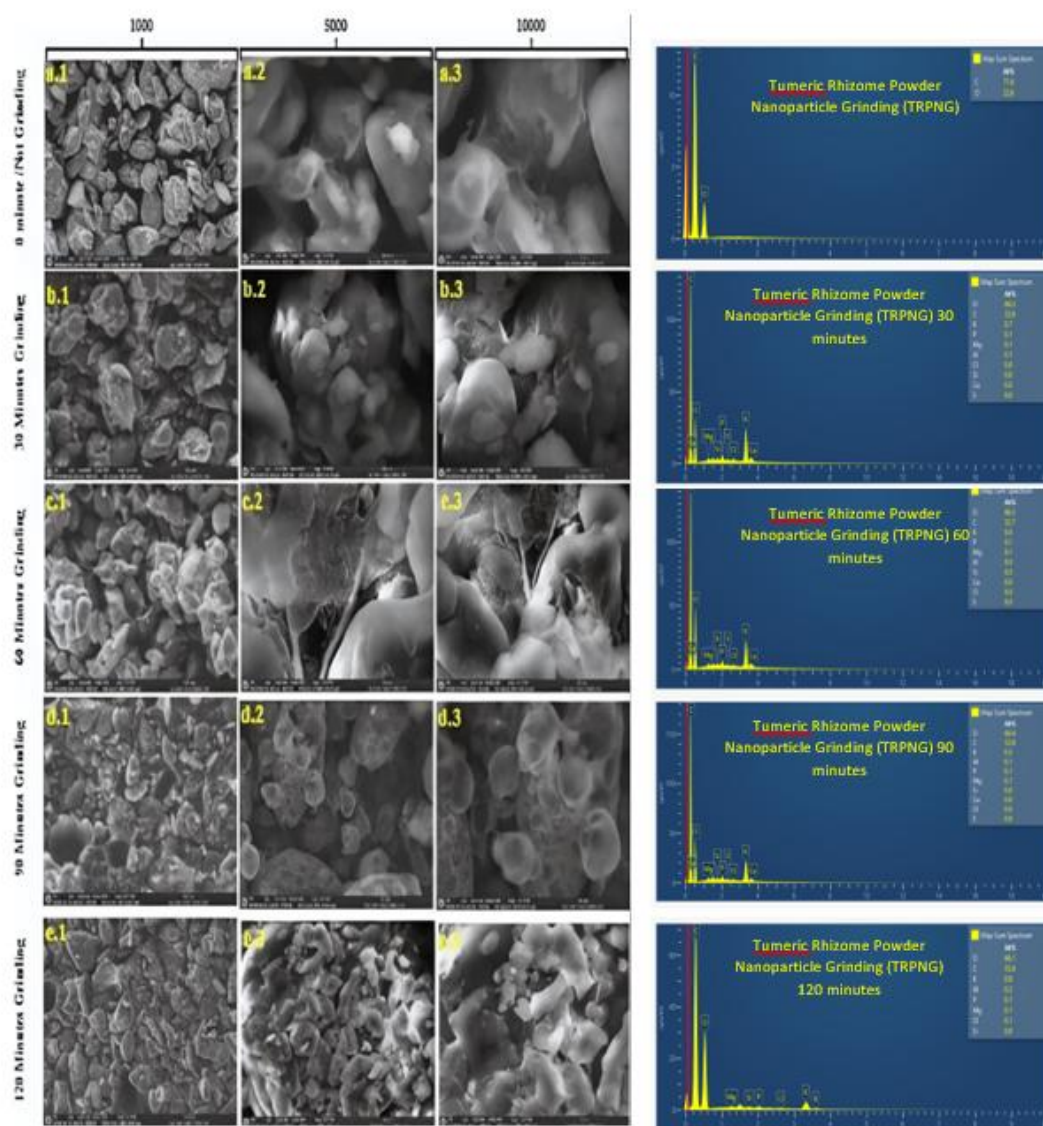


Fig. 3 Scanning Electron Microscopy (SEM)-EDX images on TRP (a1-a4), TRPNG-30 (b1-b4), TRPNG-60 (c1-c4), TRPG-90 (d1-d4), TRPNG-120 (e1-e4) at different magnifications 1000 $\times$, 5000 $\times$, and 10,000 $\times$

Prepared turmeric powder was sieved with a 100-mesh sieve and ground using a grinding machine at 800 rpm for 30, 60, 90, and 120 minutes. The outer structure of *Curcuma longa* L nanoparticles was then analyzed using SEM-EDX at 1000 $\times$, 5000 $\times$, and 10,000 $\times$ magnifications.

Fig.3 (a1-a4), shows that particles were initially large, irregular, and formed rough aggregates, consistent with Belwal (2022), who observed large block structures and low porosity in untreated herbal materials [40]. With short grinding durations, there was a marked reduction in particle size, as large aggregates break into smaller fragments, supporting the findings of Kwade et al (2018), that brief grinding produces small fragments without high surface reactivity [41]. The particles were very small, and these results are consistent with the particle size characteristics (Fig. 4, TRPNG-60). They were, more uniform, and began to form spherical nanoparticles with smooth surfaces. Mackert (2023) noted that at this stage, the crystalline structure is maintained [42].

As grinding continued, some particles underwent recrystallisation or small-scale agglomeration, forming clusters due to electrostatic forces and heat from friction [43]. This re-agglomeration, as described by Nasser (2023), resulted in morphological

degradation and loss of nanoscale size when the grinding exceeded the optimal duration [44]. The best morphology—smooth, uniform nanoparticles without agglomeration—was achieved at 60 minutes.

The data highlight the importance of optimizing grinding time and speed to achieve desired particle size and morphology. Controlled, uniform nanoparticles are essential for applications in drug delivery and catalysis. These findings can be used to develop standard protocols and predictive models for grinding different materials under various conditions.

Optimizing the grinding conditions balances effective size reduction with preventing agglomeration, which can significantly impact reactivity, photoreactivity, surface area, bioavailability, and toxicity. Aggregation decreases exposed surface area, directly affecting material reactivity, especially in catalytic processes [45,46]. In pharmaceuticals and medicine, agglomeration's effects are complex and must be carefully considered during research and development [47].

In Fig. 3 (a4-e4), the Energy Dispersive X-ray (EDX) spectrum of the samples is presented, showing the elemental composition analysis. The X-axis of the EDX spectrum represents the energy of detected X-ray signals in keV, with each element displaying characteristic peaks at specific values—for example, carbon around 0.28 keV, oxygen at 0.52 keV, magnesium at 1.25 keV, and chlorine at 2.62 keV. Higher energies correspond to elements with larger atomic numbers. The Y-axis, measured in cps/eV, shows the intensity of the detected signal, reflecting the relative abundance of each element on the sample surface [44]. The EDX graph illustrates the elemental spectrum for turmeric rhizome powder after different grinding times 0, 30, 60, 90, and 120 minutes).

Based on the EDX data, the primary elements identified in the turmeric powder samples were oxygen (~66.1–66.4%) and carbon (~32.4–32.8%), which are indicative of organic compounds such as curcuminoids and essential oils. Minor elements detected include potassium (0.5–0.9%), phosphorus (0.1–0.2%), and trace amounts of magnesium, aluminum, and chlorine (~0.0–0.1%), while silicon, calcium, and sulfur were not detected. The potassium content decreased from 0.7% at 30 minutes to 0.5% at 90 minutes, then rose to 0.9% at 120 minutes, suggesting that grinding duration can influence the degradation or transformation of mineral compounds in turmeric powder [44].

Table 3. EDX (Energy Dispersive X-ray Spectroscopy) Results of Turmeric Rhizome Powder

Type Sample	O	C	K	P	Mg	Al	Cl	Si	Ca	S
Turmeric Rhizome Powder	22.6	77.4	-	-	-	-	-	-	-	-
Turmeric Rhizome Powder Nanoparticles (TRPNG) 30 Minutes	66.3	32.6	0.7	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Turmeric Rhizome Powder Nanoparticles (TRPNG) 60 minutes	66.3	32.7	0.6	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Turmeric Rhizome Powder Nanoparticles (TRPNG) 90 minutes	66.4	32.8	0.5	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Turmeric Rhizome Powder Nanoparticles (TRPNG) 120 minutes	66.1	32.4	0.9	0.2	0.1	0.1	0.1	0.0	-	-

Analysis using EDX assessed changes in the surface elemental composition of turmeric rhizome powder at various grinding times. Initially, unground turmeric showed two dominant elements: carbon (77.4%) and oxygen (22.6%), reflecting the organic makeup of curcuminoids, essential oils, and lignocellulosic fibers [24]. The absence of elements like Si, Ca, and S suggests negligible inorganic contaminants. After 30 minutes of grinding, the carbon content dropped sharply to 32.6%, while oxygen rose to 66.3%, indicating increased surface exposure of polar functional groups such as $-\text{OH}$, $-\text{COOH}$, and $-\text{C}=\text{O}$ [1]. Oxygen levels remained stable (66.1%–66.4%) through 120 minutes, suggesting a plateau in surface exposure.

Minor elements like potassium (K), magnesium (Mg), phosphorus (P), aluminium (Al), and chlorine (Cl) appeared after 30 minutes of grinding but at low concentrations ($<0.5\%$). Minor variations in these elements may indicate microstructural changes during processing. These findings serve as a basis for evaluating how processing impacts the quality and stability of turmeric's active compounds [44].

From 0 to 120 minutes, no significant elemental changes occurred after the first 30 minutes, indicating maximum element exposure is reached quickly. This supports Alsaud & Farid (Alsaud & Farid, 2020), who noted that mechanical processing enhances surface area and compound accessibility only up to a certain limit. Therefore, 30 minutes is considered optimal for maximizing elemental exposure in the turmeric rhizome powder for characterization or further nanoparticle formulation.

3.1.3. Particle Size Analyzer (PSA)

Particle size influences the physicochemical properties, storage stability, binding of active substances, and bioavailability [48,12]. In their study found that curcumin nanoparticles had smaller average diameters than those in earlier studies [12,49,50]. The following table presents particle size measurements for the turmeric powder and nanoparticle powders at different grinding durations..

The figure illustrates that grinding turmeric rhizomes for 30 to 60 minutes sharply decreases particle size from 377.32 nm to 117.16 nm representing a 69.96% size reduction. This sharp reduction suggests that the initial milling period (0–60 minutes) is characterized by rapid fracture propagation along crystalline grain boundaries and amorphous domains. After 60 minutes, particle size increases again, thus indicating that 60 minutes was the optimal duration for achieving the smallest particle size. A small particle size is crucial for improving surface area, dissolution rate, and curcumin bioavailability in nanotechnology applications. Grinding for only 30 minutes was insufficient for nanoparticle formation, while prolonged grinding (90–120 minutes) led to nanoparticle re-agglomeration driven by increased surface energy, van der Waals attractive forces, and electrostatic interactions at the nanoscale interface. The dramatically increased specific surface area generates thermodynamically unfavourable surface energy states that drive particle coalescence [49,51,30]. The data indicate that 60 minutes is optimal, producing the smallest particle size (117.16 nm).

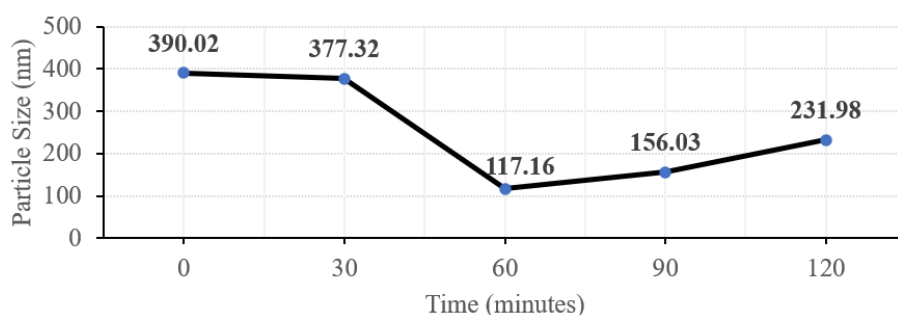
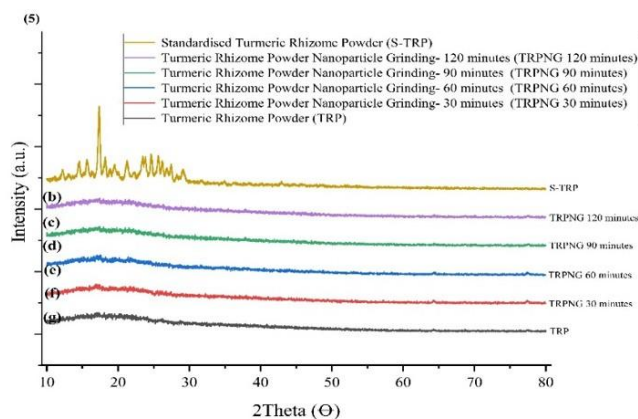
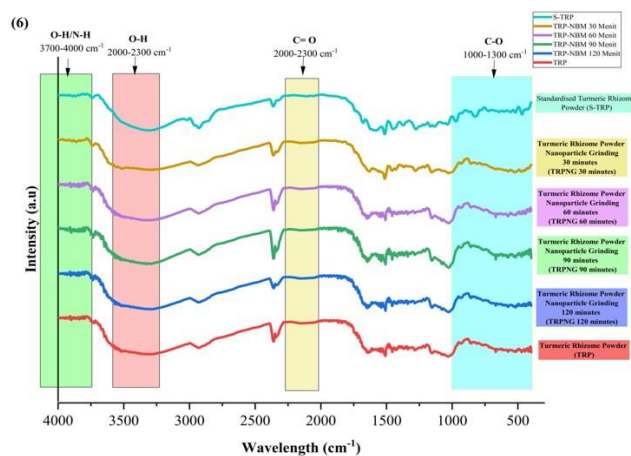


Fig. 4. Effect of grinding duration (0,30,60,90, 120 minutes) on particle size (nm)

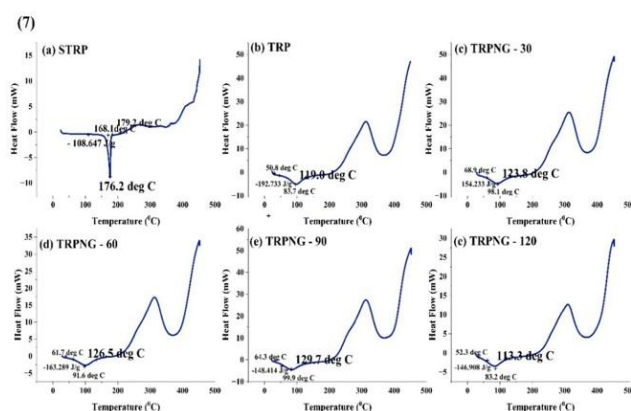
Changes in particle size, shape and chemical composition can affect the crystal structure, chemical bonds and thermal stability of the material. The crystal structure is influenced by particle size and the amorphous/crystalline nature of the compound (Fig. 5). Similarly, functional groups are influenced by the phytochemical content and elements (Fig. 6), and the thermal stability can also be influenced by size, morphology and other types of compounds (Fig. 7).



(a)



(b)



(c)

Fig. 5.6.7. Structural and chemical characterization of the turmeric samples. (b) X-ray diffraction (XRD) patterns of standard S-TRP, TRPNG-120,90,60,30, TRP (5a,5b,5c,5d,5e), (c) FTIR spectra of S-TRP, TRPNG-30,60,90,120, TRP (6a,6b,6c,6d,6e), DSC (Differential Scanning Calorimetry) S-TRP, TRPNG-30,60,90,120, TRP (7a,7b,7c,7d,7e)

3.1.4. XRD (X-ray diffraction pattern)

The X-ray diffraction (XRD) patterns of the samples are shown in Fig. 5. XRD testing of powder, nanoparticles, and ground samples at 30, 60, 90, and 120 minutes was performed by measuring the intensity of X-rays diffracted at specific angles (2θ).

The X-axis (2θ) represents diffraction angles ranging from 10° to 80° , while the peak positions correspond to crystal plane spacing based on Bragg's law. The Y-axis (intensity in arbitrary units) indicates the diffracted X-ray intensity at each angle, where higher peaks reflect greater crystallinity of the sample.

In the standard turmeric powder (Fig. 5a), sharp and high-intensity peaks signified a dominant crystalline phase. As the grinding duration increased from 30 to 120 minutes (5a-5b), the peaks became broader and flatter, showing decreased crystallinity and increased amorphous character. This aligns with the principle that grinding deforms crystal structures [7].

After 120 minutes of grinding (5e), there was a notable reduction in crystallinity due to the destruction of the crystalline phase and increased surface area [27]. However, excessive grinding risks thermal or oxidative degradation, potentially lowering active compound content [52, 28]. Broad, flat peaks indicate a shift toward amorphous material, with grinding beyond 60 minutes (5d) significantly reducing crystallinity and promoting amorphization in turmeric rhizome powder.

3.1.5. Differential Scanning Calorimetry (DSC)

Fig. 5b presents the DSC (Differential Scanning Calorimetry) thermogram alongside the thermal analysis results of the samples. In the DSC graph, the X-axis (horizontal) represents temperature ($^\circ\text{C}$), indicating the heating temperature applied to the sample during analysis in the range of 50°C – 500°C . This is intended to indicate the temperature at which thermal events (melting point or decomposition) occur. The vertical (Y) axis represents heat flow (mW), the amount of heat flow (amount of heat released or absorbed) by the sample. Positive values indicate heat release (exothermic), and negative values indicate heat absorption (endothermic) [53,54].

Curcumin, known as diferuloylmethane ($\text{C}_{21}\text{H}_{20}\text{O}_6$), is a polyphenol with a molecular mass of 368.37 g/mol and a melting point of 183°C [55,56]. Standard curcumin powder displays characteristic reference transitions at 108°C and 129°C (Fig. 7a), serving as quality control points. Heat flow stabilizes after 200°C , indicating high stability following water evaporation [57]. In the unground turmeric rhizome powder, endothermic peaks appeared at 57°C (water/volatile transition) and 171°C (initial curcumin degradation), with a significant exothermic peak around 400°C reflecting oxidation and complete degradation of the organic matrix.

For the nanoparticle powder (TRPNG) ground-for 30 minutes, the endothermic peaks shifted to 45 – 163°C (Fig. 7b) with more negative heat flow, suggesting increased surface area and enhanced heat absorption. Exothermic peaks above 400°C correspond to degradation of complex phenolic compounds [57,58]. After 60 minutes of grinding, the endothermic peak shifted lower 42 – 135°C (Fig. 7c) and became sharper, indicating a finer structure, faster heat absorption, and potential for quicker moisture and active compound loss. At 90 minutes, the initial endothermic temperature dropped to 39°C (Fig. 7e), making vaporization and degradation easier [57,58].

The graph highlights a notable phase transition below 100°C , linked to volatile compound release. TRPNG-120 minutes showed transitions at 82°C and 113°C , pointing to increased chemical fragmentation and the impact of longer grinding on thermal stability [57,58].

Grinding for 30 minutes produces sharper, deeper peaks than grinding for longer durations, suggesting that moderate grinding helps preserve curcumin and bioactive components, supporting better thermal stability. In contrast, excessive grinding reduces transition temperature and damages active compounds. Nanoparticles from 30-minute grinding show the most favorable transition temperature and peak profiles in DSC analysis (Fig. 7b), indicating optimal grinding duration. These findings can be used as validation criteria to assess post-grinding quality [59,30].

3.1.6. Fourier Transform Infrared Spectroscopy (FTIR)

In Fig. 5 (5c), the FTIR spectra of the samples are presented, showing the functional group analysis. FTIR visualization is displayed with the X-axis representing wavenumber (cm^{-1}) and the Y-axis showing absorption intensity. Some important spectral regions in peak analysis of chemical compounds in FTIR testing include 3700 – 3000 cm^{-1} ($-\text{OH}$, $-\text{NH}$ groups), 2000 –

2300 cm^{-1} (C=O), and 1000–1300 cm^{-1} (C–O). The FTIR spectrum shows the presence of characteristic groups of curcumin (OH, C=O, and C–O) [60].

In the 3700–3000 cm^{-1} region, absorption bands correspond to O–H and N–H stretching vibrations, indicating the presence of phenols, alcohols, and other polar compounds. Most samples displayed these bands, but a decrease was noted after 120 minutes of grinding, suggesting changes in hydrogen bonding or loss of volatiles. Rohman (2015), associate broad –OH bands with phenolic compounds like curcumin and its derivatives [60].

Between 2000–2300 cm^{-1} , the C=O group shows stretching vibrations, characteristic of curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Grinding samples show shifts in position and intensity when compared to the standard and unground powder, indicating hydrogen bonding interactions, partial degradation, or structural alterations from mechanical pressure. Schneider (2015), reported that such shifts may signal curcumin degradation due to heat and pressure during grinding. In the 1000–1300 cm^{-1} region, C–O group vibrations (alcohol, ester, phenol) were present across samples, but intensity slightly dropped with 120 minutes of grinding, indicating a reduced polar compound content likely from thermomechanical degradation [61]. Nanoparticle powders ground for 30 and 60 minutes displayed patterns similar to standard curcumin, while 90 and 120 minutes of grinding led to decreased –OH and C=O intensity, reflecting bioactive compound loss from excessive grinding. These findings suggest 60 minutes as the optimal grinding duration to preserve curcuminoid structural integrity.

The integration of these complementary analytical platforms provides a comprehensive holistic mechanistic understanding of the nano-transformation process. The convergence of findings across these techniques substantially strengthens the scientific rigor and translational significance of the present work, positioning it at the forefront of pharmaceutical nanotechnology research.

3.3 Biological Activity

3.3.1. Antibacterial Activity

The antibacterial activity of the turmeric powder and nanoparticle samples is presented in Fig. 8, including inhibition zone testing and correlation analysis of bacterial inhibition performance over 24 hours.

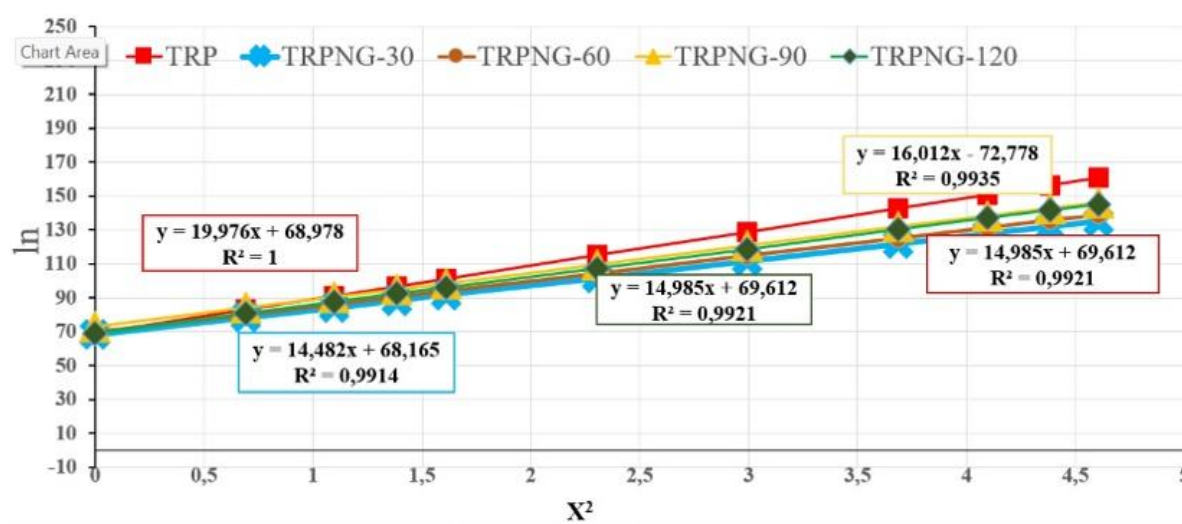


Fig. 8. Antibacterial activity and statistical analysis of the correlation coefficient of the regression equation for the minimum bacterial inhibition zone within the first 24 hours for the nanoparticle powders with grinding durations of 30, 60, 90, and 120 minutes

Table 4. The Effect of Turmeric Rhizome Grinding Duration at 24 Hours on the Bacterial Inhibition Zone of *S. aureus*

Milling Variation	Zone of Inhibition (mm)	Concentration µg/ml											P-value
		1	2	3	4	5	10	20	40	60	80	100	
Milling - 0 minutes	Mean	8.3	9.11	9.54	9.83	10.0	10.7	11.3	11.9	12.2	12.5	12.6	0.00 0*
	Std.Dev	0.0163	0.0183	0.0216	0.0163	0.0141	0.021	0.0245	0.0082	0.0183	0.0216	0.0082	
Milling - 30 minutes	Mean	8.26	8.84	9.18	9.39	9.56	10.08	10.5	11.03	11.29	11.47	11.61	0.00 0*
	Std.Dev	0.008	0.01	0.01	0.01	0.01	0.0082	0.013	0.013	0.013	0.013	0.01	
Milling - 60 minutes	Mean	8.35	8.95	9.28	9.51	9.69	10.21	10.7	11.1	11.4	11.62	11.79	0.00 0*
	Std.Dev	0.016	0.012	0.014	0.016	0.008	0.018	0.014	0.014	0.008	0.018	0.014	
Milling - 90 minutes	Mean	8.53	9.16	9.50	9.74	9.92	10.47	10.9	11.48	11.76	11.95	12.10	0.00 0*
	Std.Dev	0.0183	0.0141	0.0294	0.0141	0.0141	0.0183	0.0141	0.0141	0.0141	0.0141	0.0183	
Milling - 120 minutes	Mean	8.32	8.99	9.35	9.61	9.79	10.36	10.9	11.41	11.71	11.91	12.05	0.00 0*
	Std.Dev	0.0141	0.0183	0.0141	0.0141	0.0163	0.0183	0.0183	0.0163	0.0141	0.0163	0.0163	

*One-way ANOVA

The grinding duration has a direct impact on antibacterial effectiveness, with optimal particle size promoting better release of bioactive compounds like curcumin into the medium. Maximum inhibition zones were observed with 30–60 minutes of grinding, enhancing diffusion. However, the effectiveness declined at 120 minutes, consistent with Li and Ibrahim (2025), who found that over-processing nano-sized particles can reduce stability and biological activity. Data on the average inhibition zones of *S. aureus* from day 1 to day 7 show that turmeric rhizome powder ground for 30 and 60 minutes produced the most significant effects, with statistically significant differences ($p < 0.05$) in inhibition zones at different grinding durations [62,63].

Determination of Minimum Inhibitory Concentration (% MIC)

The determination of the Minimum Inhibitory Concentration (MIC) is measured based on the following equation:

$$\ln(\text{MIC}) = \ln \left[\frac{x^2}{4Dt} \right] \dots \dots \dots (1)$$

MIC: Minimum Inhibitory Concentration

x : Inhibitory Diameter

D : Diffusion Coefficient

t : Diffusion Time

Equation (1) is then derived as:

$$\ln(c) = \ln\left[\frac{c_0}{(MIC)}\right] - \frac{x^2}{4Dt} \dots \dots \dots (2)$$

[27,52].

For the turmeric rhizome powder, and nanoparticle powders ground for 30, 60, 90, and 120 minutes, correlation coefficients ($R^2 = 1, 0.9914, 0.9921, 0.9935, 0.9908$) with high correlation coefficients (R values) indicated a strong linear relationship between sample concentration and inhibition of *S. aureus* (Fig.6). The intercepts ($\ln MIC$) for each sample (TRP, TRPNG-30, TRPNG-60, TRPNG-90, TRPNG-120) corresponded to MIC values (3.20%, 0.905%, 0.960%, 1.06%, 1.50%), with lower MICs representing greater antibacterial potency. For instance, turmeric rhizome nanoparticle powder ground for 30 minutes achieved the lowest% MIC% (0.905%), indicating the highest effectiveness, likely due to increased release of bioactive compounds during grinding [64].

Several past studies have defined MIC values below 20% as acceptable, with Alves et al. [65]. Further categorizing MICs as highly active (<1%), strong (1–5%), moderate (5–10%), and weak (10–25%). MICs above 20% are considered insignificant for antimicrobial potential or drug development [66,67]. In this study, TRPNG-30 minutes achieved MIC of 0.905% at 24 hours, placing it in the highly active category (Fig.8).

The MIC reflects the minimum concentration needed to inhibit bacterial growth, with smaller values signifying stronger antibacterial activity. These results align with Wysocka et al (2021) [68]. (Wysocka et al., 2021), who reported that curcumin demonstrates significant antimicrobial effects against *S. aureus* after 18–24 hours of incubation. The data show statistically significant differences in the MICs across grinding durations, supporting the conclusion that optimal grinding time enhances the antibacterial efficacy of turmeric rhizome powders and nanoparticles [64, 66].

Mechanism of Action of Turmeric Powder on *S. aureus* Bacteria

The antibacterial activity of turmeric powder is believed to involve cell membrane damage, leading to disruption, leakage of cell contents, and changes in bacterial morphology. This aligns with earlier studies indicating that the antibacterial effects of curcumin are due to membrane disruption and inhibition of protein synthesis [69,70].

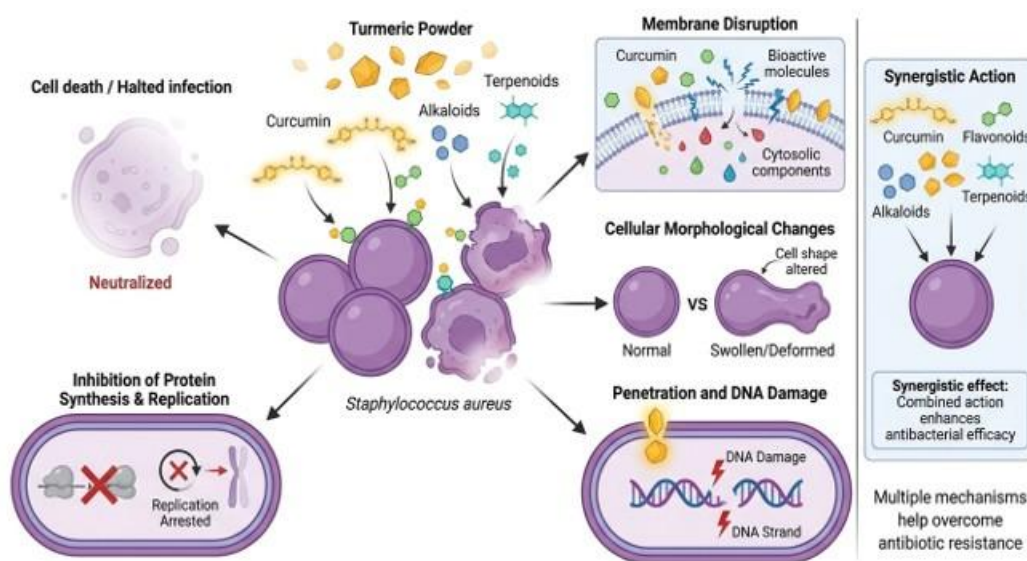


Fig. 7. Illustration Of The Mechanism Of Action Of Turmeric Powder Against *S. Aureus*

Turmeric particles disrupt bacterial replication and protein synthesis by penetrating the cell wall and compromising its structure. Once inside, they damage bacterial DNA, resulting in cell death and halting infection, thereby neutralizing *Staphylococcus aureus* by targeting vital cellular processes [69,71].

Curcumin is a key contributor due to its high concentration, but flavonoids, alkaloids, and terpenoids also play important roles through synergistic effects. These bioactive compounds work together to boost turmeric's antibacterial potency and help address antibiotic resistance by acting through multiple mechanisms [70].

4. CONCLUSION

TRPNG-30 minutes was selected as the best candidate for use as a raw material in the production of pharmaceutical formulations. This conclusion is based on the results of a comprehensive evaluation of physical characteristics and antibacterial activity, which demonstrated the greatest potential; although its physical characteristics were comparable to those of TRPNG-60, *in vivo* testing represents a higher level of validation than the initial characterisation. The simultaneous application of multiple characterization platforms, enabling comprehensive multi-analytical integration to systematically track mechanochemical transformation provides a methodologically rigorous and scientifically complete characterization framework rarely achieved in single studies. Multianalytical characterization of nanoparticle re-agglomeration kinetics contributes fundamental knowledge to the field of pharmaceutical mechanochemistry.

Based on the overall characterization results for the TRPNG samples ground for 60 minutes, the majority of the results were optimal. However, in the bacterial inhibition zone assessment, the best results were obtained with (TRPNG - 30 minutes (12.95 mm), with a % MIC of 0.905%. Therefore, TRPNG-30 minutes was prioritized for further evaluation as a material for nanoparticle gel production. This study demonstrates, through FTIR, that curcuminoid chemical integrity is fully preserved during mechanochemical amorphization without chemical degradation (Fig.5-7), addressing a critical knowledge gap regarding the safety and efficacy of top-down nanoparticle fabrication for thermosensitive phytochemicals

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AUTHOR CONTRIBUTIONS

Bina M. Girsang, Yuandani, Hakim B, Tengku I. Hanum: Conceptualization, Investigation.

Bina M. Girsang, Hakim B, Yuandani: Methodology, Laboratory Analysis.

Bina M. Girsang, Muhammad Rusda, Tengku I. Hanum: Data Curation, Formal Analysis.

Bina M. Girsang, Yuandani, Hakim B: Supervision, Writing, Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The authors declare that there is no financial, personal, or professional conflict of interest that could be perceived as influencing the research, the interpretation of the data, or the results presented in this manuscript. This study, titled " Mechanical Grinding-Derived Turmeric Rhizome Nanoparticles (*Curcuma longa* L.): Characterization and Comparative Antibacterial Efficacy" was conducted with full adherence to academic and ethical integrity.

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