



Effect of the Alcoholic Extract of *Curcuma longa* on Acne-Causing Bacteria: *Staphylococcus aureus*, *Propionibacterium acnes* (Cutibacterium acnes), and *Staphylococcus epidermidis*

Saja Abbas Mazher*

Department of Forensic Techniques, Najaf Technical Institute, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

ABSTRACT

The present study investigated the antibacterial activity of the alcoholic extract of *Curcuma longa* (turmeric) against acne-causing bacteria. Acne vulgaris is a common irritating skin condition in adolescents and adults, comprising pimples, comedones, pustules, and cysts due to chronic inflammation of hair follicles and associated sebaceous glands. Visual manifestations of acne often result in psychological complications such as depression and social morbidity. Three bacterial species, including *Staphylococcus aureus*, *Propionibacterium acnes* (*Cutibacterium acnes*) and *Staphylococcus epidermidis*, were used in this study. The alcoholic extract was prepared using (75%) ethanol, and evaluated through the disc diffusion method to measure inhibition zones. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Results revealed that the turmeric extract exhibited antibacterial activity against all tested strains, with inhibition zone increasing proportionally with the extract concentration. The highest concentration (100 mg/mL), the mean inhibition zones for *S. aureus*, *P. acnes*, and *S. epidermidis* were (39.21±0.75 mm), (33.04±1.06 mm), and (42.30±0.90 mm) respectively. Positive control (tetracycline 10 µg) produced inhibition zones of (40.00±0.71 mm), (34.45±0.06 mm), and (43.45±0.21 mm) against *S. aureus*, *P. acnes*, and *S. epidermidis*, respectively. The MIC for all tested bacteria was (25 mg/ml), while the MBC was (100 mg/mL). The findings suggest that the alcoholic extract of *Curcuma longa* may serve as a natural alternative or complementary treatment for acne, particularly against *S. aureus* and *S. epidermidis*.

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E-mail addresses:

sja.abbas.inj@atu.edu.iq

* Corresponding author

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INTRODUCTION

Acne vulgaris is one of the most common inflammatory skin disorders, predominantly affecting adolescents and young adults, and imposing a significant physical and psychological burden. Acne is recognized as a complex, multifactorial condition involving excessive sebum production (seborrhea), follicular hyperkeratinization, bacterial colonization,

and immune-mediated inflammatory responses within the skin [1]. Microorganisms residing in sebaceous glands and hair follicles play a critical role in acne pathogenesis [2]. Recent studies indicate that skin microbiome dysbiosis contributes significantly to disease development, rather than the mere presence of a single bacterial species. Among these, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* are considered the major etiological agents contributing to the development and persistence of acne lesions [3].

Propionibacterium acnes is considered the most important microbial factor in acne pathogenesis. It is a Gram-positive, non-motile, facultative anaerobic bacterium that predominantly inhabits lipid-rich sebaceous follicles, where it utilizes the lipid environment to grow and proliferate [4]. Although *P. acnes* is also present on healthy skin, molecular studies have revealed significant strain diversity within this species, identifying three major phylotypes: IA, II, and III. Strains of phylotype IA1 are closely associated with severe inflammatory lesions, whereas other phylotypes are more commonly found on healthy skin or non-pathogenic colonization [5].

P. acnes contributes directly to inflammation through the production of enzymes such as lipases, which hydrolyze triglycerides in sebum into free fatty acids, causing skin irritation and activation of keratinocytes. Additionally, the bacterium produces proteins such as CAMP (Christie-Atkins-Munch-Petersen) factors, proteolytic enzymes, and biofilm-forming factors, which constitute complex structures that enhance bacterial resistance to antibiotics and local immune defenses [6].

Moreover, *P. acnes* activates pattern recognition receptors such as TLR2 on keratinocytes and skin immune cells, inducing

the secretion of pro-inflammatory mediators including IL-1 β and IL-8, thereby creating a localized inflammatory environment that drives the formation of papules and acne lesions [7].

Staphylococcus epidermidis is a common commensal bacterium residing on human skin and is generally considered part of the non-pathogenic skin microbiome. Studies have indicated that this bacterium plays a dual role in acne pathogenesis. Certain strains of *S. epidermidis* can exert protective effects by producing antimicrobial compounds, such as lactic acid and Bacteriocins. Evidence also suggests that a balanced presence of *S. epidermidis* contributes to the stability of the skin microbiome and prevents the over colonization of *P. acnes* [8].

Conversely, some studies have reported an increased prevalence of specific *S. epidermidis* strains in inflammatory acne lesions, suggesting that certain genotypes may stimulate inflammation or participate in biofilm formation alongside *P. acnes*. Additionally, *S. epidermidis* can produce free fatty acids that irritate the skin, which have been observed more frequently in samples from patients with treatment-resistant acne [9].

Staphylococcus aureus is a Gram-positive bacterium capable of surviving across a wide temperature range (6.5–46°C) and can form colonies on mucous membranes and skin within 24 hours, with diameters reaching up to 4mm and a size of approximately 0.5–1.0 μ m. It possesses a single cell wall that leads to the formation of characteristic golden yellow pigmented colonies [10]. *S. aureus* is recognized as an opportunistic pathogen, easily colonizing facial skin and causing clinical manifestations such as inflamed wounds, pus formation, and abscesses due to rapid bacterial proliferation [11].

Curcuma longa L. (turmeric), is a prominent medicinal and aromatic plant

belonging to the family Zingiberaceae. It is a perennial herb characterized by thick, yellow-orange rhizomes, which are the primary part used for medicinal and dietary purposes. Turmeric predominantly grows in tropical regions of Southeast Asia, especially India, which remains the largest global producer and exporter [12].

Turmeric contains a wide range of bioactive compounds, primarily curcuminoids, which are polyphenolic compounds responsible for its distinctive yellow color. The main curcuminoids include curcumin, demethoxycurcumin, and bisdemethoxycurcumin [13]. The rhizome also contains volatile oils, with major constituents such as turmerone, atlantone, and zingiberene, which contribute to turmeric's characteristic aroma and biological activity [14].

Medicinally, turmeric has been extensively studied and used in traditional medicine systems, such as Ayurveda and Chinese medicine, for treating inflammation, wounds, liver and gastrointestinal disorders, and various skin conditions. Curcumin strongly inhibits inflammatory processes by modulating molecular pathways, thereby reducing the secretion of pro-inflammatory cytokines such as IL-6 and TNF- α [15].

Additionally, curcumin exhibits potent antioxidant properties by inhibiting the production of reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes such as catalase and superoxide dismutase. Turmeric also demonstrates broad-spectrum antimicrobial activity against bacteria and fungi, mainly by disrupting cell membranes and inhibiting protein synthesis [16].

Turmeric extract can be effectively used in topical dermatological treatments due to its anti-inflammatory and antibacterial properties, particularly in acne management. It helps reduce sebum secretion, inhibits the

growth of acne-causing bacteria such as *S. aureus* and *P. acnes*, and contributes to faster tissue healing and the maintenance of skin microbiome balance [17].

MATERIALS AND METHODS

Bacterial Samples:

Bacterial isolates of *Staphylococcus aureus*, *Propionibacterium acnes*, and *Staphylococcus epidermidis* were obtained from dermatology laboratories as pre-identified and characterized strains by microbiology specialists from dermatology laboratories, using skin swabs collected from patients with inflammatory acne. The purity of the isolates was confirmed by subculturing on selective and differential media, such as Mannitol Salt Agar for *S. aureus* and *S. epidermidis*, and Anaerobic Agar for *P. acnes*. The isolates were subsequently stored on Nutrient Agar slants at 4°C until use and were reactivated before experimentation to ensure viability and diagnostic accuracy [18].

Preparation of Alcoholic Extract of Turmeric

The rhizomes of *Curcuma longa* were thoroughly washed with distilled water and shade-dried at room temperature (25-28°C) for ten days. The dried rhizomes were then ground into a fine powder using an electric grinder. A quantity of 50 g of turmeric powder was placed in a glass flask containing 250 mL of 75% ethanol. The flask was sealed and intermittently shaken for 72 hours at room temperature. The extract was subsequently filtered through Whatman No.1 filter paper and concentrated in an oven at 40°C to obtain an alcoholic extract [19]. To prepare the different concentrations of the alcoholic extract, the crude extract was diluted with sterile ethanol to obtain the specific weight-to-volume (w/v) concentrations. A (25%) concentration was obtained by diluted one portion of the extract in three portions of

ethanol, while a (50%) concentration was prepared by diluted one portion of the crude extract in one portion of ethanol. Whereas the (75%) concentration was obtained by diluted three portions of the extract in one portion of ethanol, and the (100%) concentration the crude extract was used undiluted. After preparation, all concentrations were then stored in a dark, tightly sealed container at 4°C until further use [20].

Preparation of Bacterial Suspensions

The bacterial isolates were reactivated by culturing on Nutrient Agar and incubating at 37°C for 24 hours. Bacterial suspensions were then prepared in sterile physiological saline, and the turbidity was adjusted to match the 0.5 McFarland standard, corresponding approximately to 1×10^8 CFU/mL [21].

Antibacterial Activity test

The antibacterial activity of the turmeric alcoholic extract was assessed using the disc diffusion method. Sterile nutrient agar was poured into Petri dishes and allowed to solidify. The agar surface was then inoculated with the prepared bacterial suspension. Wells with a diameter of 6 mm were created in the agar and filled with different concentrations of the extract (25, 50, 75, and 100 mg/mL). Prior to inoculation, the bacterial suspension's turbidity was verified and calibrated to match the 0.5 McFarland standard by using a

spectrophotometer at (600 nm). Plates were incubated at 37°C for 24 hours for aerobic bacteria and 48 hours for anaerobic bacteria by using anaerobic jars with GasPak systems. The inhibition zones around the wells were measured in millimeters to evaluate antibacterial activity, with tetracycline (10 µg) serving as a positive control and ethanol as a negative control [22].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC values were determined using the serial dilution method in Nutrient Broth. Gradual concentrations of the turmeric extract (25-100 mg/mL) were added to tubes containing standard bacterial suspensions. Following incubation, the lowest concentration showing no visible turbidity was recorded as the MIC. Subculturing from these tubes onto Nutrient Agar plates was then performed to determine the MBC, defined as the lowest concentration that completely inhibited bacterial growth [23].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 26 software. All data were presented as mean $\pm$ standard deviation. One-way ANOVA test and post-hoc Fisher's LSD were used for comparative analysis with significance level of ($P < 0.05$)

RESULTS and DISCUSSION

The results of the present study demonstrated that the alcoholic extract of

Curcuma longa exhibited significant antibacterial activity $p<0.05$ against acne-causing bacteria, including *Staphylococcus aureus*, *Propionibacterium acnes*, and *Staphylococcus epidermidis*. The antibacterial effect was evaluated using the disc diffusion

method to determine the inhibition zones at different concentrations of the extract (25, 50, 75, and 100 mg/mL). The mean diameters of the inhibition zones (Mean $\pm$ SD) and statistical significance for *S. aureus*, *P. acnes*, and *S. epidermidis*, are shown in Table 1.

Table 1. Mean $\pm$ SD of diameter inhibition zones (mm) of alcoholic turmeric extract against acne-causing bacteria

Bacteria	mg/mL (MIC)	mg/mL (MBC)
<i>S. aureus</i>	25	100
<i>P. acnes</i>	25	100
<i>S. epidermidis</i>	25	100

Values are expressed as mean $\pm$ SD of three independent experiments

It is evident from Table 1 that the alcoholic extract of turmeric exhibited significant antibacterial activity ($p<0.05$) against all tested isolates. The degree of inhibition varied among bacterial species, and the extract's efficacy increased with higher concentrations. The largest inhibition zones were observed at 100 mg/mL, where *Staphylococcus epidermidis* (42.30 $\pm$ 0.90 mm) showed the highest sensitivity, followed by *Staphylococcus aureus* (39.21 $\pm$ 0.75 mm), and *Propionibacterium acnes* (33.04 $\pm$ 1.06 mm). These findings, support by statilcal analysis in Table 1, indicating a dose-dependent antibacterial effect of the turmeric extract against *S. aureus*, *P. acnes*, and *S. epidermidis*.

All bacterial species showed inhibitory responses even at the lowest tested concentration (25mg/mL), whereas complete bactericidal effects were observed at (100 mg/mL), as shown in Table 2.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of alcoholic turmeric extract against acne-causing bacteria.

Concentratio ns of extract (mg/mL)	<i>S. aureus</i>	<i>P. acnes</i>	<i>S. epidermidis</i>	Value
25	13.20 $\pm$ 1.31	14.57 $\pm$ 0.40	0.65	P<0.05
50	22.80 $\pm$ 0.72	21.27 $\pm$ 0.31	18.65 $\pm$ 0.48	P<0.05
75	33.54 $\pm$ 0.86	28.96 $\pm$ 0.41	27.05 $\pm$ 0.46	P<0.05
100	39.21 $\pm$ 0.75	33.04 $\pm$ 1.06	34.54 $\pm$ 0.90	P<0.05
Tetracycline	40.00 $\pm$ 0.71	34.45 $\pm$ 0.06	42.30 $\pm$ 0.21	P<0.05
Ethanol	0	0	43.45 $\pm$ 0	

The results of the present study demonstrated that the alcoholic extract of

Curcuma longa exhibited antibacterial and anti-inflammatory activities against a range of

acne-causing bacteria, including *S. aureus*, *P. acnes*, and *S. epidermidis*. The findings showed a gradual increase in the diameters of the inhibition zones with increasing extract concentrations. Specifically, the Gram-positive bacterium *S. aureus*, exhibited mean inhibition zones of (13.20 ± 1.31 , 22.80 ± 0.72 , 33.54 ± 0.86 , and 39.21 ± 0.75 mm) at extract concentrations of (25, 50, 75, and 100 mg/mL) respectively ($p < 0.05$), confirming that higher concentrations of bioactive compounds were associated with stronger antibacterial activity. These results agree with previous research reporting high efficacy of alcoholic turmeric extracts against *S. aureus* [24,25]. The antibacterial effect of the extract can be attributed primarily to its rich content of curcuminoids, particularly curcumin, as well as phenolic compounds, terpenoids, and tannins, which collectively exert antimicrobial, antioxidant, and anti-inflammatory activities. Tannins, for instance, bind iron more effectively than gallic acid, which limits the availability of iron required for essential bacterial functions, including DNA synthesis and other critical cellular processes, thereby preventing bacterial proliferation [26].

Furthermore, the inhibition of essential bacterial enzymes, including DNA gyrase and β -lactamase, increases the susceptibility of *S. aureus* to antibiotics, suggesting that turmeric extract may serve as a natural adjunct to conventional antibacterial therapies [27]. The high mean inhibition zone of (39.21 ± 0.75 mm) at (100 mg/mL) indicates that the extract's efficacy is comparable to standard antibiotics, highlighting its potential clinical relevance in treating skin infections where *S. aureus* plays a major role [28].

The antibacterial activity against *S. epidermidis* was even more pronounced, with mean inhibition zones of (18.65 ± 0.65 , 27.05 ± 0.48 , 34.54 ± 0.46 and 42.30 ± 0.90 mm) at extract concentrations of (25, 50, 75, and 100 mg/mL), respectively. This observation indicates that increasing the concentration of bioactive compounds enhances the biological

response of this bacterium to the extract. These findings are in accordance with studies [29,30]. The greater sensitivity of *S. epidermidis* relative to *S. aureus* may be attributed to structural differences in their cell walls, with *S. epidermidis* possessing a relatively simpler peptidoglycan layer that allows easier penetration of hydrophobic compounds such as curcuminoids and phenolics, while lacking certain defensive enzymes present in *S. aureus* [31]. Curcumin's interference with cell membrane proteins and destabilization of the peptidoglycan wall, combined with the extract's volatile oils such as turmerone, enhances the antibacterial response [32].

Additionally, the extract's ability to inhibit biofilm formation enhances its efficacy against *S. epidermidis*, as biofilms typically confer resistance to antibacterial agents. Ethanol, as the extraction solvent, efficiently recovers these non-polar bioactive compounds, allowing deeper penetration into bacterial membranes, thereby enhancing the extract's broad-spectrum antibacterial activity [33]. The maximum mean inhibition zone of (42.30 ± 0.90 mm) at (100 mg/mL) shows antibacterial activity comparable to standard antibiotics used for treating follicular inflammation and other skin infections caused by *S. epidermidis* [34].

Regarding the anaerobic bacterium *P. acnes*, a major causative agent of acne vulgaris, the alcoholic turmeric extract demonstrated significant antibacterial activity ($p < 0.05$), with mean inhibition zones of (14.57 ± 0.40 , 21.27 ± 0.31 , 28.96 ± 0.41 , and 33.04 ± 1.06 mm) at (25, 50, 75, and 100 mg/mL), respectively. These results align with those reported in another research [35,36]. Emphasizing the extract's ability to reduce bacterial adhesion and biofilm formation. The dual antibacterial and anti-inflammatory effects of curcumin are especially relevant for acne management, as it inhibits *P. acnes* growth while reducing pro-inflammatory

cytokines such as IL-8 and TNF- α in keratinocytes [37].

Furthermore, curcumin decreases the production of lipase and protease enzymes secreted by *P. acnes*, which are responsible for local inflammation in sebaceous glands [38]. The slightly lower susceptibility of *P. acnes* to the extract, compared to *S. aureus* and *S. epidermidis*, may be attributed to its lipid-rich waxy layer, which limits compound penetration, as well as its anaerobic growth, which reduces direct exposure to oxygen-soluble active constituents [39].

The antibacterial and anti-inflammatory mechanisms of the turmeric extract are multifaceted. The curcuminoids-curcumin, demethoxycurcumin, and bisdemethoxycurcumin interfere with bacterial cell wall biosynthesis, alter membrane permeability, and induce leakage of intracellular components, leading to bacterial cell death [40]. Phenolic compounds and terpenoids, including α -turmerone and zingiberene, further contribute by generating oxidative stress in bacterial cells and inhibiting critical metabolic pathways [14]. Alkaloids and tannins also act synergistically, disrupting protein synthesis and inhibiting bacterial reverse transcriptase enzymes required for replication, these bioactive compounds collectively inhibit fatty acid synthesis in *P. acnes*, while exhibiting antioxidant activity that mitigates inflammatory responses in the skin [41].

The results of the MIC and MBC tests further demonstrated that *S. epidermidis* and *S. aureus* are more sensitive to the extract than *P. acnes*, which can be attributed to structural differences among Gram-positive bacteria and the lipid-rich envelope of *P. acnes* [39].

CONCLUSIONS

The alcoholic extract of *Curcuma longa* L. exhibited strong antibacterial activity against *S. epidermidis*, *S. aureus*, and *P. acnes*, with the antimicrobial effect increased

proportionally with concentration and reaching maximum mean inhibition at (100 mg/mL). At this highest concentration, the mean inhibition zones were comparable to those of produced by standard antibiotic tetracycline, while MIC and MBC results showed higher sensitivity of *S. epidermidis* and *S. aureus* compared to *P. acnes*, likely due to structural differences in the cell walls. The antibacterial properties of the extract are mainly attributed to curcumin, phenolic compounds, and terpenoids, which disrupt bacterial protein synthesis and DNA replication, suggesting that *Curcuma longa* extract serves as a promising natural alternative for acne treatment and related skin infections.

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CONFLICT OF INTEREST

The author acknowledge that this study is free of any conflict of interest.

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