

# Antibacterial activity of curcumin against Lebanese clinical isolates of *Staphylococcus aureus*

## Abstract

The speed of emergence of resistance to newly introduced antimicrobial agents, by strains of common pathogens, has necessitated the search for newer medications. The use of medicinal plants and their extracts has become popular and promising. The methanol extract from *Curcuma longa*, a plant that has long been used by villagers, who noted its therapeutic properties, was tested, in this study, for its antibacterial activity against local, multiresistant strains of *Staphylococcus aureus*. The disc agar and well agar diffusion methods showed that the plant extract was able to inhibit the growth of the tested strains at different concentrations, revealing the genetic diversity of these strains. The emergence of resistant strains to the extract during the experiments, demonstrated the “drug fast” characteristic of the organism. Further studies are needed to conclude whether an active compound in the extract can be used in developing a new antibacterial agent.

**Keywords:** antibacterial effect, curcumin, *S. aureus*, medicinal plants, methanol extraction

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Patrick Abou Raji El Feghali, Riwa Ibrahim,  
Tarek Nawas

Department of Natural Sciences, Lebanese American University,  
Lebanon

**Correspondence:** Tarek Nawas, Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Beirut, Lebanon, Tel +9611 8676 18, Email [tnawas@lau.edu.lb](mailto:tnawas@lau.edu.lb)

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## Introduction

Antimicrobial agents are some of the most widely used therapeutic agents worldwide. The use of antimicrobials made medical procedures such as organ transplant, cancer chemotherapy, and major surgery relatively safe.<sup>1</sup> The treatment of bacterial infections was made possible by the discovery of antibiotics, antibacterials that work by either killing bacteria or inhibiting their growth. However, the misuse and mishandling of antibiotics and other antimicrobials by patients as well as health care professionals resulted in the emergence and spread of resistance.<sup>1,2</sup> Microorganisms that develop resistance show decreased sensitivity to antimicrobials, rendering them ineffective in preventing and treating bacterial, fungal, parasitic, and viral infections. Another important concern is the fact that most of those agents are often associated with adverse side-effects on the host, increasing the risk of complications that could sometimes be deadly.<sup>3</sup> Those issues, along with several others, provoked the attention of researchers towards traditional treatment. Medicinal plants are of particular interest.<sup>2,4</sup> Plant oils and extracts are being extensively tested for their potential use as alternative and safe remedies for the treatment of many infectious diseases.<sup>5,6</sup> Turmeric, also known as curcumin is also an example. Turmeric is scientifically called *Curcuma longa*, a plant that belongs to the *Zingiberaceae* family.<sup>2,7,8</sup> Its rhizomes are thick and fleshy, elongated in shape and surrounded by a rough segmented skin. It has a yellowish-brown color with a pale orange color on the inside.<sup>3,9</sup> The length of the root varies from 2.5cm to 7cm, and the diameter of the main root is generally around 2.5 cm. The plant is native to Southern Asia, and is widely cultivated in the warm areas of the world namely in India, as well as other countries including China, Taiwan, Sri Lanka, Peru and Australia.<sup>7,11</sup> *C. longa* is used for several purposes in medicine, cosmetics, food flavoring and textile industries.<sup>4,7</sup> It has been shown to exhibit major pharmacological activities which include antimicrobial, hepatoprotective, antioxidant, antitumor, wound healing and antiviral effects.<sup>4,7,8</sup> Furthermore, it displays important therapeutic effects against cardiovascular, pulmonary, metabolic, neurodegenerative and autoimmune

diseases.<sup>2,10</sup> The plant is also prescribed for the treatment of a wide range of health issues related to the digestive tract.<sup>11</sup> Curcumin has also shown significant antibacterial activity against several strains of both Gram positive and Gram-negative bacteria.<sup>9</sup> *Staphylococcus aureus* is one of the Gram-positive strains that was reported to be susceptible to curcuma.<sup>9</sup> *Staphylococcus aureus* is a common cause of severe infections in health facilities (nosocomial infections) and the community.<sup>3</sup> It can cause various infections including infective endocarditis (IE), bacteremia, skin and soft tissue, osteoarticular, and pleuropulmonary infections.<sup>9</sup> Over the years, *S. aureus* has evolved and developed multiple strategies to evade the human immune system and to resist antibiotic treatment (MRSA).<sup>9</sup> The aqueous extract of *Curcuma longa* has shown antibacterial activity against *S. aureus*.<sup>2,11</sup> The methanol extract of turmeric also revealed an inhibitory effect against *S. aureus*.<sup>3,9</sup> The study of hexane and ethanol turmeric extract showed antibacterial activity against 13 bacterial species including *S. aureus*.<sup>9</sup> Antimicrobial susceptibility tests of different fractions of *C. longa* rhizome extract against *S. aureus* clinical isolates showed that all fractions of *C. longa* rhizome are highly active against standard and clinical isolates of *S. aureus* showing zone of inhibition ranges between 9mm and 21mm. Further observations revealed that the benzene extract was least active, showing zone of inhibition of about 9mm at the concentration of 50mg/ml while methanolic extract was most active showing zone of inhibition of about 19mm at the concentration of 50mg/ml.<sup>3</sup> Curcumin was also reported to exhibit an inhibitory effect on methicillin-resistant *S. aureus*.<sup>9</sup> The purpose of this study was to test the effect of different concentrations of methanol extract of curcumin on local clinical multiresistant strains of *S. aureus*.

## Methods

### Plant used

Samples of *Curcuma longa* were purchased from a local farmer's shop, definitively identified by our reference botanist and then immediately sent to the microbiology laboratory for processing.

## Bacterial isolates

The bacterial isolates used in the study were three multiresistant clinical isolates of *Staphylococcus aureus*, provided by the Clinical Microbiology Laboratory of the Lebanese American University Medical Center- Rizk Hospital (LAUMC - RH). Randomly, the isolates were named: *Staphylococcus aureus* isolates 1, 2 and 3. Gram staining and standard biochemical methods were performed to confirm the identity of the test organisms.<sup>12</sup>

## Preparation of the methanol extracts

Two hundred grams of *Curcuma longa* roots were weighed using a mass balance, chopped and mixed with 80% methanol in a sterile blender. The extract was then transferred to several Erlenmeyer flasks and kept in an orbital shaker for 1 week. The extracts were then filtered using vacuum assisted Millipore filtration.

## Disc agar diffusion

A volume of 50µL of the extract was added to standard and sterile filter paper discs to be used in the experiment. The Muller Hinton Agar (MHA) plates which were prepared as recommended,<sup>13</sup> where seeded with an inoculum of the tested strains, that was adjusted to have a turbidity equivalent to a 0.5 McFarland standard. Four of the prepared discs were inserted properly on the surface of each of the seeded plates, after which the plates were incubated at 35 °C for 24 hours. Any zone of inhibition of growth, around any of the discs, was then measured using a caliper. The reported results were the average of the diameters measured of the zones of inhibition of growth around the discs inserted on each plate.

## Well agar diffusion

Muller Hinton Agar (MHA) was prepared as recommended.<sup>13</sup> Using a cork borer wells were introduced in the middle of the MHA plates that were seeded with the test organisms (adjusted to have a

**Table 1** Diameters, in millimeters, of the zones of inhibition of growth of the three *Staphylococcus aureus* isolates, using different concentrations of the methanol extract of *C. longa*. C: Control with only methanol\*; concentration at which few and scattered resistant colonies observed within the zones of inhibition

	C	50 µL	100µL	150µL	300 µL	350µL	400µL	450µL	500µL	550µL	600µL
<i>S. aureus</i> (isolate 1)	0	13.7*	0	8.0*	22.7*	24.0*	25.1*	27.4*	29.0	31.0	34.0
<i>S. aureus</i> (isolate 2)	0	12.4*	0	0	27.0*	28.7*	30.4*	31.7*	35.7*	40.4*	43.7
<i>S. aureus</i> (isolate 3)	0	10.4*	0	9.7*	24.4*	30.3*	38.4*	41.0*	48.3*	51.0	57.7

## Discussion

Whereas a volume of 50µL was enough to show a defined zone of inhibition using the disk agar diffusion method, no inhibition was observed when a volume of 100µL was added in the agar well diffusion method. This is attributed to the fact that the kinetics of diffusion of the antimicrobial agents into the agar from a surface application (disk agar diffusion assay) is different from diffusion through the agar (well agar diffusion assay) when the antimicrobial is placed into a well.<sup>14</sup> This could also be explained by the dependence of the well diffusion assay on several factors including the thickness of the agar.<sup>15</sup> In addition to that, the fact that the mixture contained different constituents exhibiting different diffusion rates could also be

turbidity equivalent to a 0.5 McFarland standard). The different volumes of extract to be tested were added to these wells, after which the plates were incubated at 35 °C for 24 hours. The plates were then checked for the appearance of zones of inhibition of growth and a caliper was used to measure their diameters. The reported results were the averages of the diameters measured for each of the zones of inhibition of growth that surrounded the wells for each concentration of the tested extract.

## Results

When the disk diffusion assay was performed with 50µL of *C. longa* extract added on the disk, inhibition of the three different isolates used in this experiment were noted (Table 1). When 100µL of extract was added when performing the well agar diffusion assay, no inhibition zone was detected for any of the *Staphylococcus aureus* isolates used (Tables 1). However, when the volume was raised to 150µL, *C. longa* extract was able to inhibit *Staphylococcus aureus* isolates 1 and 3 but not *Staphylococcus aureus* isolate 2 (Table 1). Moreover, when the volume of extract added increased gradually from 300µL to 600µL, inhibition zones were recorded for the three different *Staphylococcus aureus* isolates. Small colonies of *Staphylococcus aureus* isolate 1 in the zone of inhibition appeared on the plates where the volume of extract added ranged from 50µL to 450µL (Table 1). However, these colonies disappeared when the volume of extract added reached 500µL, 550µL and 600µ. Small colonies of *Staphylococcus aureus* isolate 2 in the zone of inhibition also appeared on the plates where the volume of *C. longa* extract added ranged between 50µL and 550µL, but a volume of 600µL was able to inhibit the growth of these small colonies. On the plates of *Staphylococcus aureus* isolate 3 where the volume of *C. longa* added increased from 50µL to 500µL, small colonies appeared in the zone of inhibition. Nonetheless, these small colonies disappeared when the volume of extract added reached 550µL and 600µL.

an explanation of the results obtained. In order to obtain comparable and reproducible results, the techniques used were standardized, as much as possible, by having the inoculum with a density equivalent to 0.5 McFarland, a thickness of agar of 4mm in all used plates, and the use of Muller Hinton Agar as recommended.<sup>13,16</sup> A clear and defined zone of inhibition was observed starting with the addition of 150µL of *C. longa* extract. As expected, the diameter of the zone of inhibition increased with increasing the volume added until reaching the largest diameter when a volume of 600µL was added (Table 1). Genome sequencing projects for multiple isolates of a bacterial pathogen are of considerable scientific value because the generated data reveals not only their gene content but also the conservation and variability of genes in different strains of the organism. The diversity of *S. aureus*

is mainly due to polymorphisms that occur in genomic islands, which carry many virulence and antibiotic resistance determinants.<sup>17</sup> These differences were demonstrated by the results which showed that at the same volume of *C. longa* extract added for the three *S. aureus* isolates tested, different diameters of the zone of inhibition were observed indicating that each of the strains possessed certain genetic characteristics that rendered it either more or less susceptible to the antibacterial compound(s) found in the natural extract. These differences also accounted for the fact that different volumes of extract were needed to inhibit the appearance of resistant colonies within the zone of inhibition of growth of the three *S. aureus* isolates used.

*S. aureus* is one of the Gram-positive strains that is susceptible to curcumin-mediated inhibition.<sup>18</sup> However, over the years, *S. aureus* has evolved and developed multiple strategies to evade the human immune system and to resist antibiotics treatment and is thus recognized as a drug fast organism.<sup>19,20</sup> One of the forms of resistance of *S. aureus* against *C. longa* was revealed by the appearance of small colonies inside the zones of inhibition using different concentration of the methanol extract of *C. longa* (Table 1). To confirm that these colonies were indeed resistant varieties of the organism, single colonies were isolated from each of plates in which growth was seen within the zones of inhibition, their identities reconfirmed and they were used to run the set of experiments again. The results confirmed our presumption and these resistant strains were able to grow even at high concentrations of the *C. longa* extract.

## Conclusion

The results confirm that *Curcuma longa* has an effective antibacterial activity against *Staphylococcus aureus*. However, in order to use this characteristic to develop pharmaceuticals, the active antibacterial compound in the extract should be identified and purified. This will be later followed by using proper biological assessment assays, dosage formulations, and clinical studies to establish safety, efficacy and the pharmacokinetic profile of any prospective new drug. These steps are important to evaluate the potential of the extract to cause any harm to the host or to the normal flora.<sup>21</sup> The fact that *Curcuma longa* is a natural plant whose extract is often used by people without considerable complications, indicates that it is very promising that it may potentially be used in vivo without dangerous side effects. The possibility of incorporating it in common disinfectants must also be considered.

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## Conflict of interests

The authors declare that there is no conflict of interest.

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