

# The Combination Effect of Curcumin on the Antibacterial Activity of Antibiotics

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## Abstract:

The emergence of multi-drug resistant bacteria to common antibiotics and their rapid increase are major health problems; many antibiotics have become less and less effective against pathogenic bacteria, therefore there is an urgent need to search for new antibacterial substances especially from natural sources. Many studies showed that herbs may positively affect microbial susceptibility to antibiotics. In this study, the combination effect of curcumin with Amoxicillin against different pathogenic bacteria (*S. aureus*, *Streptococcus*, *E. coli*, *Pseudomonas*, and *Proteus*) was investigated, using well diffusion method. The results indicate that curcumin has a synergistic, antagonistic and indifference effect when added to different concentrations of Amoxicillin against pathogenic bacteria. Synergistic response was 70%, indifference 20%, and antagonistic was 10%. The results revealed that curcumin improved the bactericidal effect of the antibiotic and has potentials in combination therapy against different pathogenic bacteria.

## Introduction:

Infectious diseases remain a major cause of death, as they account for one – third of all deaths in the world. The discovery of antibiotics was an essential part in combating bacterial infections, but during the last few decades microbial resistance to drugs has grown remarkably because of many factors among which is the inappropriate prescription and usage of antibiotics. [1]. Many pathogens developed modes of resistance to the antibiotics they were susceptible to: Streptococci group A resistance to Sulfonamides, Multi-Drug Resistant (MDR) Pneumococci, Methicillin-Resistant *Staphylococcus aureus* (MRSA), resistant Gram-negative enteric bacilli, and most recently, MRSA showed reduced sensitivity to Vancomycin, the only remaining drug that effectively kills MRSA.

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Today, clinically important bacteria are characterized by multiple antibiotics resistance, making them difficult to treat. [2,3] The rate of emergence of antibiotic resistant bacteria is not matched by the rate of development of new antibiotics to combat them, antibiotic that work today may not work tomorrow. [4] The era of microbes resistant to all known antibiotics has approached. However, the emergence of bacterial resistance to antibiotics and its dissemination are major health problems, leading to treatment ineffectiveness for a large number of drugs. [5] Consequently, there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy. Recently, some edible natural products and food ingredients have been reported to enhance the antibacterial activity of different antibiotics. [6,7,8]

In this study, we investigated the effect of curcumin on the antibacterial activity of one of the important broad-spectrum antibiotics, Amoxicillin, against different pathogens: *Staphylococcus aureus*, *Streptococcus*, *E. coli*, *Proteus*, *Pseudomonas aeruginosa*. Curcumin as a food ingredient has drawn the attention of scientists because of its intensive pharmaceutical properties. [9,10,11] It has been reported to have an extremely good safety profile and no toxicity observed when taken at doses as high as 12gm/day, which make it an attractive choice for many disease therapies. [12,13,14]

### **Material and Methods:**

#### **Curcumin preparation:**

##### **Ethanol extraction method was used to prepare Curcumin.**

About 20g of turmeric powder was weighed in a dried clean 100mL beaker, and then 30mL of 70% Ethanol were added to the weighed beaker. The mixture was transferred to a 100mL separatory funnel and stirred for 20 min, then it was filtered and the filtrate was evaporated to dryness. Finally, the residues were weighed, Curcumin percent extract was calculated and sub-inhibitory concentration of curcumin (500µg/ml) was prepared.

#### **Biological Assay to Evaluate Combined Effect:**

Antimicrobial activity was tested by agar well diffusion method.

Different test strains were used: *S. aureus*, *Streptococcus*, *E. coli*, *Proteus*, and *Pseudomonas aeruginosa* Mueller – Hinton agar (MHA Oxoid) was prepared, sterilized and cooled to 45°C, then distributed into Petri dishes. Inoculum of 10<sup>6</sup>cfu/ml for each strain according to McFarland turbidity standards was inoculated by swabbing on the Mueller-Hinton agar plates, and then wells of 5mm diameter were prepared in each agar plate.

The wells were loaded with 100µl of different concentrations of Amoxicillin alone: 125,250, 500, 1000µg/ml. Similar experiment was carried out to determine the combined effect; each concentration of Amoxicillin was mixed with sub- inhibitory concentration of curcumin (500µg/ml). Plates were

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incubated at 37°C for 24hrs, and at the end of the period, the inhibition zones were measured in mm.

### Results and Discussion:

The diameters of inhibition zones (mm) with and without curcumin against test strains are shown in table 1 and table 2 respectively. 500µg/ml curcumin is a sub-inhibitory concentration and it was chosen to guarantee that the effect produced was due to the combination, not to the curcumin itself. [15] There were increases in the antibacterial activity of Amoxicillin in the presence of curcumin against test strains. The highest increase in area was observed for 250µg/ml Amoxicillin against *Streptococcus* (55.5%). The lowest increase in inhibition zones was observed for 500µg/ml Amoxicillin against *Escherichia coli* (4.8%) Curcumin showed some antagonistic effect on the antibacterial activity of this antibiotic (125µg/ml) against *Escherichia coli* and *Pseudomonas aeruginosa*, (14.3%) and (10%) respectively, while there was no effect of curcumin and Amoxicillin combination against *Proteus* strain which was resistant to Amoxicillin alone. In the majority of concentrations tested, curcumin significantly improved antibiotic efficiency against test strains when combined with the antibiotic, as synergistic response was 70%, indifference 20% and antagonistic was 10%.

Curcumin may inhibit efflux transporter mediated bacterial resistance to different antibiotics, [16,17] or the synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms [18,19] (*Streptococcus* 55.5%-40.9% increase in inhibition zone) either by inhibiting the cell wall synthesis or by causing its lyses or death. [20,21]

The results demonstrated that the combination of herbs with antibiotics reduced drug resistance, and curcumin as a safe natural product could also serve as a valuable agent for combination therapy due to its potential synergistic effect with antibiotics against pathogenic bacteria. [22,23,24]

(Table: 1) Antimicrobial activities of different conc. of Amoxicillin on test strains

Microorganism	Inhibition Zones (mm)			
	Conc. Of Amoxicillin (µg/ml)			
	125	250	500	1000
<i>Staphylococcus aureus</i>	24	26	27	29
<i>Streptococcus</i>	8	8	13	14
<i>Escherichia coli</i>	35	35	40	42
<i>Pseudomonas aeruginosa</i>	10	19	19	20
<i>Proteus</i>	0	0	0	0

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(Table: 2) Inhibition zones of combination of curcumin with Amoxicillin

Microorganism	Inhibition Zones (mm)			
	Conc. Of Amoxicillin ( $\mu\text{g/ml}$ )			
	125	250	500	1000
<i>Staphylococcus aureus</i>	31	36	40	42
<i>Streptococcus</i>	15	18	22	26
<i>Escherichia coli</i>	30	40	42	45
<i>Pseudomonas aeruginosa</i>	9	20	23	17
<i>Proteus</i>	0	0	0	0

(Table: 3) Percentage of synergistic/antagonistic effect of combination of curcumin with Amoxicillin

Microorganism	Percentage of Synergistic/Antagonistic Effect			
	Conc. Of Amoxicillin ( $\mu\text{g/ml}$ )			
	125	250	500	1000
<i>Staphylococcus aureus</i>	22.6	27.7	32.5	30.9
<i>Streptococcus</i>	46.6	55.5	40.9	46.1
<i>Escherichia coli</i>	-14.3	12.5	4.8	6.7
<i>Pseudomonas aeruginosa</i>	-10	5.0	17.4	17.6
<i>Proteus</i>	0	0	0	0

The number of natural extracts acting in synergy with synthetic drugs towards microbial species is large. There is a need for more studies concerning the molecular basis of synergistic interactions to understand the synergistic mechanism which is fundamental to the development of pharmacological agents to treat bacterial infections using medicinal plants which exhibit synergistic behaviour.

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Thorria R. Marzoog, Wasnaa H. Mohammed, Iman Ismail Jabbar, Rana Shamil Noori

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## تأثير مزيج من الكركمين والمضادات الحيوية كمضاد للفعالية الحيوية للبكتيريا

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رنا شامل نوري

ظهرت الأدوية المتعددة للبكتيريا المقاومة للمضادات الحيوية الشائعة ونموها السريع والمشاكل الصحية الرئيسية؛ كثير من المضادات الحيوية أصبحت أقل وأقل فعالية ضد البكتيريا المسببة للأمراض، ولذلك هناك حاجة ملحة للبحث عن مواد جديدة مضادة للبكتيريا المرضية، لا سيما من مصادر طبيعية. وأظهرت دراسات عديدة أن الأعشاب قد تؤثر بشكل إيجابي على حساسية الجراثيم للمضادات الحيوية. في هذه الدراسة، تم التحقيق في تأثير مزيج من الكركمين مع أموكسيسيلين ضد البكتيريا المسببة للأمراض المختلفة ( *S. aureus, Streptococcus, E. coli, Pseudomonas, and Proteus* )، وذلك باستخدام طريقة الانتشار. وتشير النتائج إلى أن الكركمين له تأثير متناغم أو متعاقد أو تأثير متضاد أو بدون تأثير عندما تضاف إلى تركيزات مختلفة من أموكسيسيلين ضد البكتيريا المسببة للأمراض. وكان استجابة التآزر أو التعاضد 70%، 20% بدون تأثير، وكان 10% هناك تضاد بين عمل الكركمين مع المضاد الحيوي. أظهرت النتائج أن الكركمين حسن من تأثير المضادات الحيوية القاتلة للبكتيريا ويملك مقومات في الجمع بين العلاج ضد البكتيريا المسببة للأمراض المختلفة.