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Turmeric: An effective natural antibiotic

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Abstract
The purpose of this article is to provide a brief overview on the research done regarding the antibiotic property of Turmeric and its role in treating infections by providing bacterial resistance and thus, acting as an natural antibiotic. Turmeric, a spice that has been long recognized by both the medical and scientific world for its medicinal properties. It helps in the treatment of oxidative and inflammatory conditions, arthritis, metabolic syndrome, hyperlipidemia and anxiety because of its antioxidant and anti-inflammatory properties. Curcumin, a principal bioactive substance of turmeric (*Curcuma longa* L.), is reported as a strong antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral agent. In this work, we tested curcumin's efficacy against over 100 strains of pathogens belonging to 19 species. This activity was determined by the broth microdilution method and by calculating the minimum inhibitory concentration (MIC). Our findings confirmed a much greater sensitivity of Gram-positive than Gram-negative bacteria. Similarly, the MICs of the MDR types of *Staphylococcus aureus* and *S. haemolyticus* were high. However, curcumin was effective against some species and strains: *Streptococcus pyogenes*, methicillin-sensitive *S. aureus*, and individual strains of *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Hence, turmeric can be considered as a promising antibacterial agent, but with a very selective activity.

Keywords: *Curcuma longa*, curcumin, antibacterial activity, antifungal activity, minimum inhibitory concentration (MIC)

1. Introduction
Turmeric is an herbal plant with a very long history of medicinal use, dating back nearly 4000 years and a major source of the polyphenol curcumin. Curcumin is a bioactive substance from turmeric. Turmeric helps in the treatment of exercise-induced inflammation and muscle soreness, thus enhancing recovery and performance in active people. In addition, a comparatively low dose of the complex can provide health benefits for people that do not have diagnosed health conditions.

Curcumin is a phytochemical derived from the rhizome of *Curcuma longa*. Curcumin (curcumin I, diferuloylmethane) is a dimeric derivative of ferulic acid, composed of two o-methoxyphenol rings connected by a heptadienedione chain. It has a chemical formula of $C_{21}H_{20}O_6$ and a molecular weight of 368.38 g/mol. This lipophilic polyphenol is a natural pigment with a characteristic yellow-orange color, predominantly found in the rhizomes of turmeric (*Curcuma longa* L.) from the ginger family, Zingiberaceae, native to tropical South Asia. It has both antibacterial as well as antibiofilm activity. Ingesting curcumin by itself does not lead to the associated health benefits due to its poor bioavailability. There are several components that can increase bioavailability. For example, piperine a major active component of black pepper when combined with curcumin has shown an increase in bioavailability by 2000%. Curcumin combined with enhancing agents gives multiple health benefits. Curcumin can restore bacterial susceptibility to antibiotics by inhibiting its biofilm mode of growth and making it sensitive to antibiotics *in vitro* (Karaman M, 2013) [9]. Its antibacterial action is found to be synergic with several antibiotics (Sasidharan NK, 2014) [18].

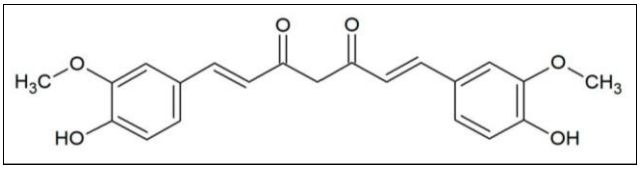

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Fig 1: Chemical structure of Curcumin.

India produces nearly 80% of the world's turmeric crop. Due to its innate qualities and high content of the important bioactive compound curcumin, Indian turmeric is considered to be the best in the world. Erode, a city in the South Indian state of Tamil Nadu is the world's largest producer and important trading center for turmeric and is also known as "Yellow City", "Turmeric City" or "Textile City". In Erode, it is not only used as the main spice but also as an ingredient in religious ceremonies. Turmeric because of its brilliant yellow color is known as "Indian saffron". Modern medicine has begun to recognize the importance of turmeric and over 3000 publications dealing with it came out in the last 25 years.

2. Mechanism

The phytochemical component in turmeric is used as a dietary spice and a topical ointment for the treatment of inflammation (H. P. T. Ammon, 1991) [7]. Curcumin is widely used in India for centuries. It has been effective against a variety of disease conditions in both *in vitro* and *in vivo* preclinical studies. Diferuloylmethane a major component of food flavoring turmeric, is being reported to be anti-carcinogenic and anti-inflammatory as well as antioxidant properties. It is insoluble in water but dissolves in acetone, dimethylsulfoxide, and ethanol. Its commercial grade contains 10–20% curcuminoids, desmethoxycurcumin, and bisdesmethoxycurcumin, they are effective as pure curcumin.

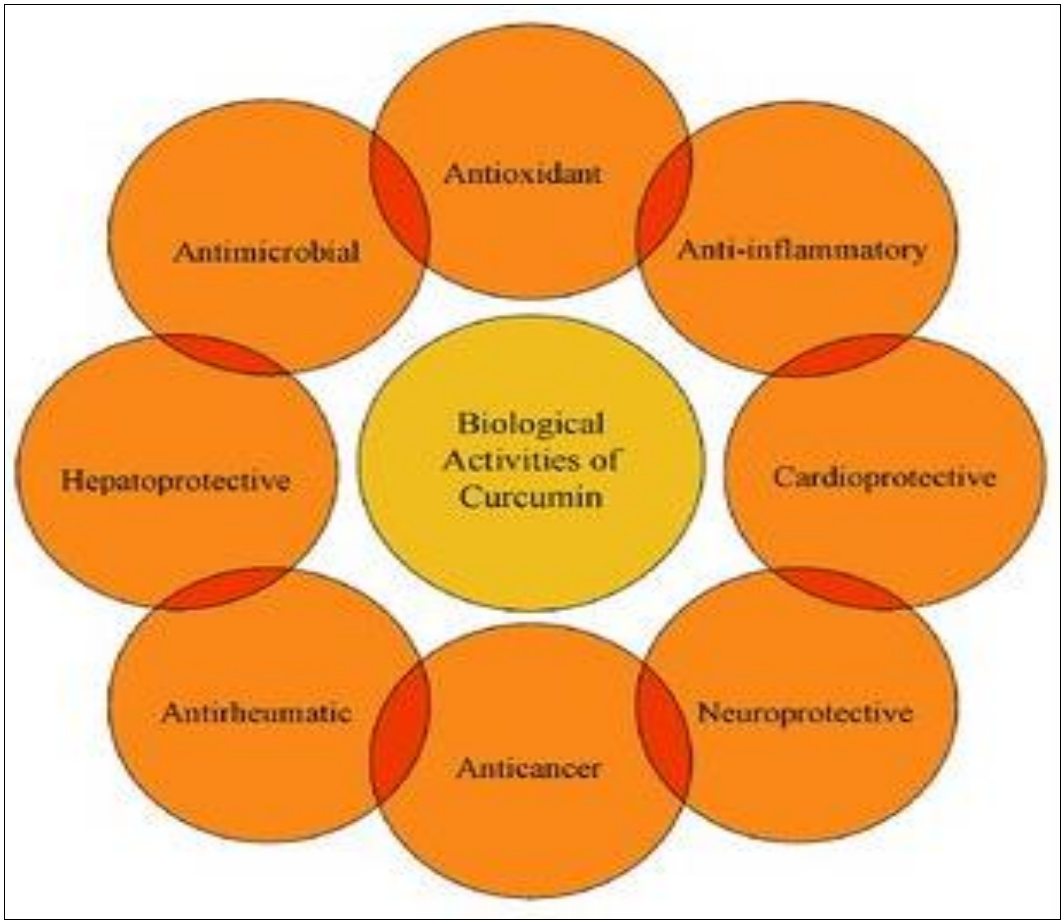

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Fig 2: Biological Activities of Curcumin

2.1. Anti-Inflammatory Activity

Both the *in vitro* and *in vivo* studies have demonstrated its effects on decreased acute and chronic inflammation. It is a potent anti-inflammatory with specific lipoxygenase and COX-2 inhibiting properties, it has inhibited Edema disorders at doses between 50-200 mg/kg body weight in mice. It is as effective as cortisone and phenylbutazone at similar doses. It also inhibited formaldehyde that induced arthritis in rats at a dose of 40 mg/kg/day. A study performed on an animal showed rheumatoid arthritis was induced by a streptococcal cell wall, intraperitoneal injection of turmeric extract containing 4 mg total curcuminoids /kg/day for four days prior to induction of arthritis, inhibited joint inflammation in both acute (75%) and chronic (68%) phases. it reduced joint inflammation by 48% in four days (Satoskar RR, 1986) [19].

2.2. Anti-microbial Activity

It has been shown that turmeric inhibits the growth of many bacterial species, pathogenic fungi, and parasites. When a study conducted on Eimeria maxima, an infection in chicks demonstrated that the diets which were supplemented with 1% turmeric resulted in a reduced intestinal lesion and they gained weight. Another study showed that the application of turmeric oil inhibited the growth of dermatophytes and pathogenic fungi in guinea pigs in a 7-day post turmeric application. It has also been found to have moderate action against the plasmodium falciparum and Leishmania and major other organisms (Dujic J, 2009) [5].

2.3. Anti-oxidant Activity

Curcumin is a powerful scavenger of oxygen free radicals, and its antioxidant activity is comparable to vitamin C and E. It protects lipids and haemoglobin from oxidation it significantly inhibits the generation of reactive oxygen species such as H_2O_2, superoxide anions, and nitric radical generation by activated macrophages. Its pre-treatment study

has shown to decrease ischemia-induced oxidative stress and changes in the heart. The measuring effect of curcumin on an inducible stress protein in an *in-vitro* study resulted in enhanced cellular resistance to oxidative lungs (Bernard GT, 1982) [3].

2.4. Anti-diabetic Activity
Effects of turmeric on cholesterol levels are decreased due to less cholesterol uptake by the intestine and increased conversion of cholesterol to bile acids in the liver (Khajehdehi P, 2012) [11].

2.5. Anti-cancer Activity
Many studies have explored turmeric's influence on carcinogenesis. It inhibits cell proliferation and tumor growth and can also able to suppress the activity of several common mutagens and carcinogens. Anti-carcinogenic effects of turmeric had been related to direct antioxidant and free radical scavenging effects, as well as their ability to indirectly increase glutathione levels by aiding in hepatic detoxification of mutagens and carcinogens (B. Aggarwal, 2003) [2].

3. Methodology
A prospective study was conducted to demonstrate assess the antimicrobial efficacy of curcumin against various strains of bacteria and yeast-like fungi. Curcumin can be considered an effective antibacterial substance, but with a selective activity. For the research, commonly found human pathogens including those causing infections of the skin, chronic wounds and mucous membranes were selected. This activity was examined by the broth microdilution method and by calculation of minimum inhibitory concentration.

3.1. Clinical Studies
Recently many studies have been done on turmeric to treat several diseases in humans. In one of these studies, the anti-mutagenic effects of turmeric were examined in 16 chronic smokers (Polasa *et al*., 1992) [16] where turmeric was given in doses of 1.5 g/day for 30 days and was found to significantly reduce the urinary excretion of mutagens in these smokers. On the other hand, in six non-smokers no change in urinary excretion of mutagens was noted.

In another study, the effect of turmeric was examined on patients with irritable bowel syndrome where 1 or 2 tablets of a standardized turmeric extract were given daily for 8 weeks, and the prevalence of irritable bowel syndrome was significantly decreased. The alcoholic extract of turmeric offered protection against BaP-induced increase in micronuclei in circulating lymphocytes of healthy individuals (Hastak *et al*., 1997) [8].

Turmeric is also useful in healing peptic ulcers. In the phase II clinical trial, 45 patients with peptic ulcers were provided with capsule-filled turmeric orally in the dose of 2 capsules (300 mg each) five times daily. After 4 weeks of treatment, ulcers were found to be absent in 48% of cases and after 12 weeks of treatment ulcer-free cases increased to 76% (Prucksunand *et al*. 2001) [17]. A double-blind trial found turmeric to be helpful for people with indigestion and stomach or intestinal ulcers but it was shown to be less effective than antacids. An ethanol extract of turmeric was found to produce remarkable symptomatic relief in patients with external cancerous lesions. In a study of 62 patients, depletion in smell was noted in 90% of the cases and reduction of itching in almost all cases. Some patients (10%) had a reduction in lesion size and pain (Kuttan R, 1987) [12].

3.2. Materials and Methods
3.2.1 Curcumin
Curcumin plant substance was dissolved in a 15% water solution of dimethyl sulfoxide (DMSO) at a final concentration of 10 mg/mL. In addition, 15% DMSO was used as a negative control.

3.2.2. Microbial Strains and Culture Media
Antimicrobial activity of curcumin was examined against growth in aerobic conditions of six species of Gram-positive bacteria (*S. haemolyticus*, *S. epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *S. pyogenes*), nine Gram-negative bacteria (*Acinetobacter baumannii*, *Klebsiella oxytoca*, *Proteus mirabilis*, *K. pneumonia*, *A. lwoffii*, *Escherichia coli*, *Serratia marcescens*, *Stenotrophomonas maltophilia*), and four species of yeast-like fungi (*Candida albicans*, *Saccharomyces cerevisiae*, *C. glabrata*, *C. tropicalis*). Mostly, six clinical strains were investigated for each microbial species. Additionally, four strains for reference, namely *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *P. aeruginosa* NCTC 6749, and *E. coli* ATCC 25922 were used as controls. From among the multidrug-resistant (MDR) bacteria, they *in vitro* tested 16 clinical isolates of methicillin-resistant *S. aureus* (MRSA), extended-spectrum β-lactamases (ESβL) positive *E. coli*, ESβL-positive *P. mirabilis*, vancomycin-resistant *E. faecalis* (VRE). The total number of strains investigated was 111 from 19 species. The clinical microbial strains were isolated from the skin and mucous membrane infections. Their drug resistance was tested according to the rule of the European Committee on Antimicrobial Susceptibility Testing. The microorganisms were grown at 35 ^{0}C for 24 h, bacteria in tryptone soy agar, and fungi in Sabouraud dextrose agar.

3.2.3. Anti-microbial Activity
The minimum inhibitory concentrations (MICs) of curcumin were determined by the broth microdilution method using 96-well plates. The *in vitro* tests were carried out according to the suggestion of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method and were described in publication [Karpi ´nski, 2019] [10]. Primarily, 90 µL of Mueller–Hinton broth was placed in each well. The following final concentrations of curcumin were derived: 5000, 4000, 3000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 µg/mL. The inoculums were manipulated to contain approximately 108 CFU/mL of microbe. Then, 10 µL of the appropriate inoculums were added to the wells, obtaining a final concentration of 105 CFU/mL. To each well, 5-diphenyl-2H-tetrazolium bromide (MTT), 10 µL of a 1% aqueous solution of 3-(4, 5-dimethyl-2-thiazolyl)-2, was added [Adamczak. *et al*., 2020] [1]. Next, the plates were incubated at 35 ^{0}C for 24 h. The MIC of curcumin was taken as the lowest concentration of this substance that restricted any visible microbial growth. The analyses were repeated two times for all strains tested in this experiment.

3.2.4. Statistical Analysis

Table 1: Antimicrobial activity of curcumin against clinical and reference strains of pathogens

Microbial Strains	Minimum Inhibitory Concentration, Mean MIC Values (μg/mL)	Median
1. Gram-positive bacteria		
Streptococcus pyogenes ($n = 6$) [1]	**31.25** (4×) [2], **62.5**, 125	31.25
S. agalactiae ($n = 6$)	2000 (3×), 3000 (2×), 4000	2500
Staphylococcus aureus ($n = 6$)	125, 250 (3×), 500 (2×)	250
S. aureus ATCC 29213	250	
S. haemolyticus ($n = 6$)	500 (6×)	500
S. epidermidis ($n = 6$)	500 (2×), 1000 (3×), 2000	1000
Enterococcus faecalis ($n = 6$)	**62.5**, 500 (3×), 1000, 2000	500
Methicillin-resistant *S. aureus* ($n = 4$)	2000, 4000, >5000 (2×)	>4500
Methicillin-resistant *S. haemolyticus* ($n = 3$)	2000, >5000 (2×)	>5000
Vancomycin-resistant *E. faecalis* ($n = 1$)	5000	
Median	500 [3] a, >5000 [4] b	
2. Gram-negative bacteria		
Acinetobacter lwoffii ($n = 3$)	125, 250 (2×)	250
A. baumannii ($n = 3$)	>5000 (3×)	>5000
Escherichia coli ($n = 6$)	500 (2×), 1000, 2000 (2×), 3000	1500
E. coli ATCC 25922	2000	
Klebsiella oxytoca ($n = 6$)	500, 1000, 2000 (2×), >5000 (2×)	2000
K. pneumoniae ($n = 6$)	2000 (5×), 3000	2000
Pseudomonas aeruginosa ($n = 6$)	**62.5**, 2000 (2×), 3000, 5000, >5000	2500
P. aeruginosa ATCC 27853 (Boston 41501)	5000	
P. aeruginosa NCTC 6749	>5000	
Proteus mirabilis ($n = 6$)	1000, 2000 (2×), 4000, >5000 (2×)	3000
Serratia marcescens ($n = 4$)	2000 (2×), >5000 (2×)	>3500
Stenotrophomonas maltophilia ($n = 1$)	>5000	
ESβL-positive *E. coli* ($n = 4$)	2000, 4000, 5000 (2×)	4500
ESβL-positive *P. mirabilis* ($n = 4$)	2000, 4000, >5000 (2×)	>4500
Median	2000 b, >4500 b	
3. Yeast-like fungi		
Candida albicans ($n = 10$)	1000, 2000, 5000, >5000 (7×)	>5000
C. glabrata ($n = 2$)	>5000 (2×)	>5000
C. tropicalis ($n = 1$)	>5000	
Saccharomyces cerevisiae ($n = 1$)	5000	
Median	>5000 b	
Negative control		
15% DMSO	>5000	

[1] Total number of microbial strains tested, [2] the number of strains with an equal sensitivity to curcumin, [3] the median for all strains from the group, [4] the median for multidrug-resistant strains. The medians with the same letter are not significantly different (post-hoc test, $p > 0.05$). All MIC tests were repeated two times. The lowest MICs are marked with a bold font.

The mean MIC results of curcumin against all microbial strains tested. For an individual group of species, medians were calculated. The Kruskal–Wallis and posthoc tests were used to determine the statistical significance of differences in the MICs of Gram-positive and Gram-negative fungi, bacteria, and multidrug-resistant bacteria. The results were considered accurate at the level of $p<0.05$.

This research exhibited a strong variation in the biological activity of curcumin according to the microbial species and strains. The minimum inhibitory concentrations (MICs) of this curcumin ranged from 31.25 to 5000 μg/mL. In some cases, curcumin was non-functioning at concentrations tested 7.8–5000 μg/mL. It was observed for 28 out of 111 strains observed (25.2%). The highest differentiation of the MICs between strains was detected for *Enterococcus faecalis* 62.5–5000 μg/mL, *Pseudomonas aeruginosa* from 62.5 to >5000 μg/mL, and *Staphylococcus aureus* from 125 to >5000 μg/mL. Only for methicillin-sensitive *Staphylococcus*, *Acinetobacter baumannii* (3 strains), *Staphylococcus haemolyticus* (6 strains) and *Candida glabrata* (2 strains) were no differences in the MIC level found. In turn, *Klebsiella pneumonia* showed a little variation in this respect. Curcumin was effective at a concentration of 2000 μg/mL and for one strain at 3000 μg/mL, for five clinical isolates of this pathogen.

Although the multidrug-resistant types of *S. aureus*, *S. haemolyticus*, *E. coli*, and *P. mirabilis* exhibited some variation in their sensitivity to curcumin, the values of MICs were not less than 2000 μg/mL. In like manner also, antifungal properties of curcumin for 14 tested strains of *Candida* spp. and Saccharomyces cerevisiae were shown to be low.

3.2.5. Results and Discussion

Various studies have shown a broad spectrum of antimicrobial properties of curcumin by various pharmacological points of action [Sharififi, 2020]. It has been studied that curcumin can stop bacterial DNA replication and alter gene expression. This mechanism can promote the cell division and proliferation of bacteria. Other research has exhibited that curcumin stimulates an apoptosis-like response in *E. coli*. In turn, founds its anti-adhesive effects against *C. albicans* biofilm making at the subinhibitory concentration of MIC/2. Moreover, it disrupts the bacterial cell membrane along with

reduces the motility of microorganisms. The *in vitro* studies have demonstrated that curcumin inhibits the polymerization of FtsZ protofilaments and inhibits the GTPase activity in the cytoskeleton of *B. subtilis*, *E. coli*, and *S. aureus* Several research showed higher activity of curcumin against Gram-positive than Gram-negative bacteria. The cells of Gram-positive bacteria are covered by a peptidoglycan layer with an additional class of lipoteichoic acids, but they do not have an outer membrane. The outer membrane of Gram-negative bacteria is largely accountable for their resistance to a large spectrum of antibiotics, such as β-lactams, colistin, and quinolones.

4. Uses of Turmeric

The use of the herbal product in infective illnesses is common in traditional medicine. Turmeric can be used as an herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, colon cancer, smallpox, chickenpox, wound healing, urinary tract infections, and liver ailments (Dixit, Jain, and Joshi, 1988) [4]. It is also used for digestive disorders to reduce flatus, jaundice, menstrual difficulties, and colic; for abdominal pain and distension, and for dyspeptic conditions including loss of appetite, postprandial feelings of fullness, and liver and gallbladder complaints. Turmeric has anti-inflammatory, choleretic, antimicrobial, and carminative actions (Mills and Bone, 2000) [15]. For arthritis 8–60 g of fresh turmeric root three times daily is been recommended and for dyspepsia dosages of 1.3–3.0 g of turmeric root are recommended (Fetrow and Avila, 1999) [6].

5. Conclusion

Curcumin is considered as an effective antibacterial agent, but with very specific activity towards individual species and strains present *in vitro* tests exhibited the strong effect of this phytochemical on some Gram-positive and Gram-negative pathogens. The present results show the health benefits of using curcumin as a widely used spice and a food additive with not only its coloring, flavoring, and preservative properties, but also with the antimicrobial effect against human pathogens. A stronger activity of curcumin against Gram-positive than Gram-negative bacteria observed in our investigations and by some other authors is very interesting from a technical point of view and requires further detailed studies because various Gram-positive pathogens, including *S. pyogenes*, *S. aureus*, *S. haemolyticus*, *S. epidermidis*, *and E. faecalis*, are still an ongoing challenge for healthcare in the treatment of infections.

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