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In-vitro study on the antibacterial and antifungal effects of different aqueous and alcoholic extracts from Curcuma Longa rhizomes

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ABSTRACT

Background: Turmeric (Curcuma longa) has been popular since ancient times in both nutritional and medicinal recipes. In modern science, studies have proven its effectiveness in inhibiting the growth of some pathogenic microorganisms, but this effectiveness depends on several factors, including the type of extraction solvents used to extract the active substances from turmeric and the method of extraction. This study investigates the antimicrobial effect of different turmeric extracts using the minimum inhibitory concentration (MIC) method.

Methods: Forty grams of dried turmeric rhizome powder were soaked in 400 ml of each solvent (distilled water, ethanol, and ethyl acetate) separately for one week. Each mixture was filtered, and the remaining solvent was evaporated. The minimum inhibitory concentration (MIC) for each crude extract was measured using microdilution broth techniques against Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 14169), and Candida albicans (ATCC 10231).

Results: The MIC values for the aqueous turmeric extract against the two tested bacteria (S. aureus and E. coli) were 0.39±0.07 µg/µl and 1.562±0.33 µg/µl, respectively. For the tested fungus (C. albicans), the MIC was 50±0.05 µg/µl. However, the ethyl acetate turmeric extract inhibited only S. aureus (MIC = 0.39±0.04 µg/µl).

Conclusion: This study revealed the antimicrobial effects of the aqueous turmeric extract against the three tested microorganisms and the antibacterial effect of the ethyl acetate turmeric extract against S. aureus. However, no inhibitory activity was observed for the ethanol turmeric extract against any tested microorganism.

Introduction

Curcuma longa, known as turmeric, belongs to the family *Zingiberaceae*. it is a perennial plant indigenous to Asia (1,2). Turmeric rhizome is the most important source of curcumin (2,3). Curcumin is a di-phenolic compound; which is responsible for the *Curcuma longa* yellow color (4). Curcumin is a coloring spice in many recipes (2). Also, curcumin is traditionally used to treat various diseases (5). Turmeric and its bioactive ingredient curcumin have therapeutic properties such as healing lesions, a decrease in blood sugar, anti-cancer effects, antioxidant effects, anti-inflammation effects as well as antimicrobial effects (4,6).

On this planet where we live, bacteria are found naturally everywhere, on and in the body of any organism, and end up in the bottoms of the oceans because they play an important role in maintaining the ecological balance (7). A small percentage of all these world's bacteria have harmful roles that cause infection and disease to humans, animals, and plants (7). For example, *Staphylococcus aureus* (*S. aureus*) is usually present on human skin, but if its balance is disturbed under any circumstance, it leads to skin infections, and by the wounds, it spreads in the body via the bloodstream and can cause serious infections in other organs like the heart valves and lungs (7). But, in general, the treatment of these infections became easier after the discovery of antibiotics which reached the peak of their discovery in the golden era (the 1940s - 1960s), and most of the antibiotics still in current use -were discovered during that period- such as tetracycline (8). Since then a gradual and clear decline in access to new antibiotics, and the rise in the misuse of antibiotics in both humans and animals over the years, for instance using antibacterial agents for viral infections, have exacerbated another problem which is bacterial resistance to certain available antibiotics (8). The phenomenon of bacterial resistance means the possibility of bacteria thriving and continuing to grow in the presence of antibiotics designed to kill them. This problem is not only difficult to treat, but there is also an increased risk of severe illness and even death due to these infections (8). Recent approximations suggest that by 2050, 10 million people will die yearly from drug-resistant bacterial infections (9). As a well-known The intestines of humans and animals normally host *Escherichia coli* which is a Gram-negative bacterium; however, due to Virulence genes of some strains of *E. coli* can cause severe morbidity and mortality in humans and animals from critical illnesses such as meningitis and urinary tract infections (10). Methicillin-resistant *Staphylococcus aureus* (MRSA) of various strains of *S. aureus* do not respond to different types of penicillin antibiotics. It causes different health issues such as furunculosis and boils. Now it is threatening public health safety by community-acquired MRSA and leads to high mortality and morbidity (11). As for the fungal level, many species of *Candida albicans* have developed various genetic and molecular mechanisms to resistant to several available antifungal agents such as Azole drugs (12).

Progress of this resistance to the existing antimicrobic drugs and growing acceptance of natural remedies, enhanced the scientists' efforts toward nature again to the level that it has become a significant trend to investigate new antimicrobial agents (13).

Human is used to using plants in all aspects of life from daily food to treatment of various diseases, which is a long bright story to be told from ancient times until now (14). Like humans and animals, plants frequently become infected by pathogenic microorganisms, thus plants have developed self-defense mechanisms to protect themselves from microbial attacks. One of these mechanisms is bioactive secondary metabolites for instance phenolic compounds, phenolic acids, flavonoids, and certain oligosaccharides which hold several pharmacological actions like anti-inflammatory, antiviral, antitumor, antioxidant, and antibacterial (15).

Several studies found that different turmeric extracts inhibit the growth of a range of bacteria such as *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli*, and *Candida albicans.* However; in many in-*vitro* studies; the same strain has shown different sensitivity towards different extracts of turmeric depending on the type of solvent (16–19).

The recent study compared the inhibitory effects of three turmeric powder extracts (aqueous extract, ethanol extract, and ethyl acetate extract) individually against two tested bacteria strains; *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*) and one tested fungus strain *Candida albicans* (*C. albicans*) by the microdilution test method.

Method

Turmeric Extracts Preparations

Dried Turmeric (*Curcuma longa*) rhizomes were bought from the local market in the Amman region in Jordan. The extraction was done according to the methods used by (13,20) with several simple changes. Briefly, the turmeric rhizomes were washed with distilled water, and then the fine crushing was done by an electric blender (Model No. MX – GX 1521, Panasonic Taiwan Co., Ltd., Taiwan). To prepare the extracts, 40 grams of turmeric rhizome powder were put in closed amber glasses. After that sequentially 400 ml of one of these solvents (distilled water, pure ethanol, and pure ethyl-acetate) was poured on the powder separately for one week. After one week of the Ultrafiltration, solvent evaporation was done (water solvent by the incubator shaker at 45 °C and alcohol solvents by rotary evaporator under reduced pressure at 40 °C for 2 days). After all, every extract was weighed and kept in closed amber glass containers (at 2˚C - 8˚C).

Sub-culturing of microorganism strains

The bacterial strains *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 14169), were grown on sterilized nutrient broth (Oxoid, UK), while *Candida albicans* (ATCC 10231) was grown on Sabouraud-dextrose broth (Oxoid, UK).
They were incubated at 37˚C for bacteria and 32˚C for fungus overnight. After that, the turbidity of each test tube was confirmed and the optical density of the suspension was read by a spectrophotometer at 600 nanometers (nm) for bacteria and 530 nanometers (nm) for fungus respectively to be sure that equals 0.08 (21,22).

Antibacterial assays

The broth microdilution method by using the 96-well microplate, was performed according to the standard method (19).
First, the liquified extract was prepared by adding 0.1 g from each extract and 60 µL DMSO (Dimethyl sulfoxide) into a sterile Eppendorf tube then the volume was completed to 1 ml with broth nutrients. After that, the components were homogenized by shaking well through the vortex. This technique was followed for three dried turmeric powder extracts (aqueous extract, ethanol extract, and ethyl acetate extract) (23).
A volume of 100 µL of nutrient broth was added to all rows of the microplate except the first one. Next, 200 µL of the extract stock solution was added to the first row.

After that, 100 µL of the first row (containing the liquified extract) was transferred to the second row. After gentle mixing of the second-row contents (100 µL nutrient broth and 100 µL liquified extract) by micropipette, 100 µL of the second row was transferred to the third row, and so on. Then 100 µL of the last row was discarded and 100 µl of the bacteria were added to each row. Accordingly, eight concentrations of the liquified extract were obtained into the rows (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg\µl). The test was repeated in triplicate on individual plates and the same procedure was done for three dried turmeric powder extracts (aqueous extract, ethanol extract, and ethyl acetate extract). Finally, all microplates were incubated aerobically at 37°C and 32°C for one day for bacteria and fungus respectively.

Antibacterial Activities

The solution in each well was pipetted gently to mix before being loaded into the instrument to measure the optical absorbance at 600 nm and 530 nm for bacteria and fungus respectively (24). 100 µL from each well of the tested plate was transferred to the new well (A1-A8) in the new 96-well microtiter plate. Then serially dilute with a multichannel pipette by transferring 20 µL down from each well to the new 8 (B1-H8) rows which previously each pour contained 180 µL of normal saline water. After mixing the contents by pipetting up and down at least twice between dilutions, 5 ul from all the dilution series from each of the wells was placed into agar plates and incubated for 20 hours After that, CFU/ml was calculated through these two equations: [Number of colonies on the Petri plate/ amount of diluted sample added to the Petri plate in ml = CFU in diluted sample (cells/ml) Then CFU in the diluted sample (cells/ml)/ dilution of the Petri plate counted= CFU in the original sample (cell/ml)]. The microbial growth was assessed by plotting the CFU vis OD and drawing a linear curve in Excel, then using linear curve equations to calculate the log CFU/mL of the tested trial (20) (25).

Statistical analysis

The absorbance is expressed as a unitless number ± SD, the MIC is expressed as a number in (µg\µl), the Colony forming unite is expressed as a number in (CFU/Ml), and the Mass of dry extract is expressed as a number in (g) using Microsoft Excel (Excel Office 365, USA) and OriginPro 9.0 64Bit.

Results

The crude extract weight after the extraction procedure of 40 g turmeric rhizome powder in every 400 ml of solvent is shown in (Table 1).

The ethanol solvent gave the highest weight of dry extract, but the ethyl-acetate gave the lowest which were respectively 2.6 g and 2 g.

Table 1. Mass of Dry Extract in Different Solvents

Solvent	Mass of dry extract (gram)
Distilled Water	2.3 g
Ethanol	2.6 g
Ethyl-Acetate	2.0 g

Anti-bacterial activity

The antibacterial effects of the three turmeric powder extracts on the two tested bacteria (Gram-positive and Gram-Negative) and one tested fungus were examined using the Biotek instrument by recording the absorbances for triplicate tests at 600 nm for bacteria

Table 2. The Antimicrobial Activities of the Different Turmeric Extracts.

The extract type	The bacteria type		The fungus type	
	S. aureus	*E. coli*	*C. albicans*	
The Aqueous Turmeric Extract	0.39± 0.07 µg\µl	1.562± 0.33 µg\µl	50±0.05 µg\µl	MIC µg\µl
The Ethanol Turmeric Extract	No-inhibition	No-inhibition	No-inhibition	
The Ethyl-Acetate Turmeric Extract	0.39 ± 0.04µg\µl	No-inhibition	No-inhibition	

The Aqueous Turmeric Extract

According to (Table 2; Fig 1). the aqueous turmeric extract could inhibit the *S. aureus* growth at MIC equal to 0.39± 0.07 µg\µl. Whereas, the MIC of *E. coli* was 1.562± 0.33 µg\µl. On the other hand, only the highest concentration of the aqueous turmeric extract inhibited the *C. albicans* growth, the MIC was 50±0.05 µg\µl.

The Ethanol Turmeric Extract

The ethanolic extract of the eight different concentrations did not exhibit any antimicrobial activities against any tested bacteria or fungus (Table 2; Fig. 2).

The Ethyl-Acetate Turmeric Extract

The eight different concentrations (50 , 25 , 12.5 , 6.25 , 3.125 ,

1.56 , 0.78 , and 0.39 µg\µl) of the ethyl-acetate turmeric extract obtained the antibacterial action against the tested Gram-positive bacterium *S. aureus*, hence 0.39 ± 0.04µg\µl was the MIC value against *S. aureus* and none of these concentrations obtained any antibacterial action against the tested Gram-negative bacterium *E. coli* or antifungal action against the tested *C. albicans* (Table 2; Fig 3).

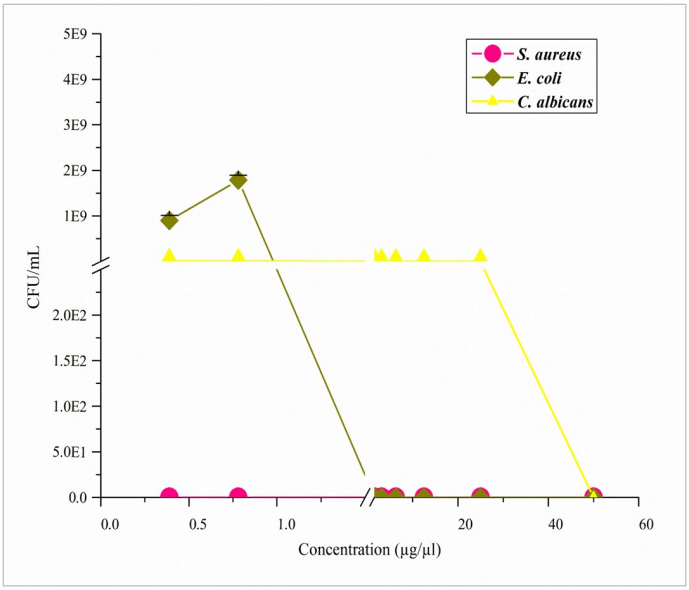


Figure 1. The Antimicrobial Activities of the Aqueous Turmeric Extract.

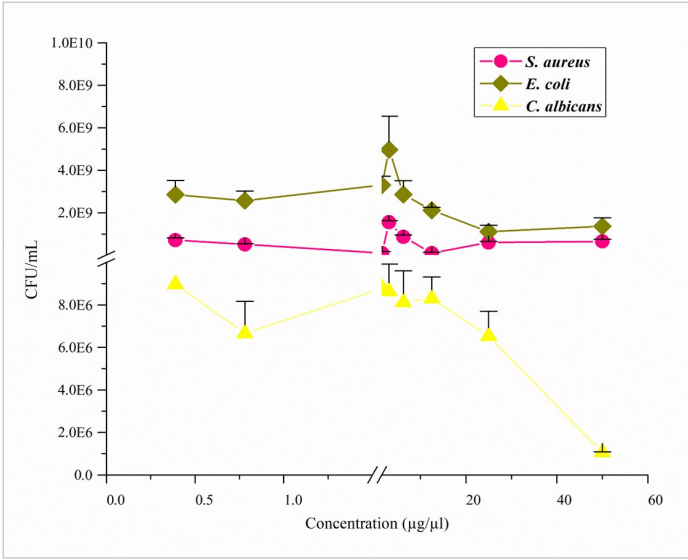


Figure 2. The Antimicrobial Activities of the Ethanol Turmeric Extract.

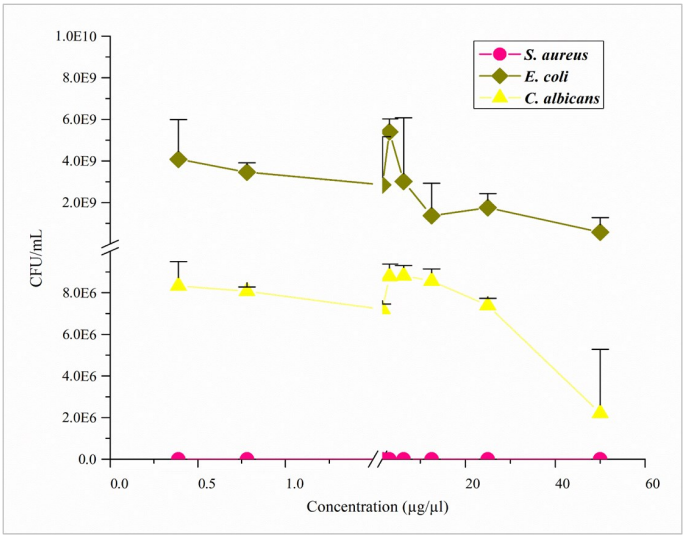


Figure 3. The Antimicrobial Activities of the Ethyl-Acetate Turmeric Extract.

Discussion

Turmeric is a sterile plant. This plant has dark yellow flowers and fleshy-ringed rhizomes (24). Turmeric contains several components with various percentages, 2–5% curcuminoid compounds, 40–70% carbohydrates, 6–8% proteins, 5–8% oils, and 3–5% other elements (26). Turmeric possesses potential medicinal propriety such as antimutagenic, anticoagulant, antifungal antifertility, and antibacterial activities (27).

Today, the spread of multidrug-resistant (MDR) has become a popular issue due to the overuse and misuse of antibiotics in different life aspects like human medical, veterinary, and agricultural. In 2019, approximately 1.27 million deaths were related to bacterial MDR according to the Global Burden of Disease (GBD) (28). The greatest cause of all these MDR bacteria, was *Acinetobacter baumannii, Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli (E. coli)*, and *Staphylococcus aureus*. These bacteria caused about 0.93 million of all death cases annually (28,29).

Thus, finding herbals-based antimicrobial agents has paid the researchers attention day by day. There are 250,000 to 50,000 various plants on the earth, now scientists have completely recorded just around 10% of them that have different therapeutic benefits in the laboratory. From these ten percent, black, coriander, onion, ginger, basil pepper, and turmeric. Among these mentioned plants, turmeric shows maximum antimicrobial efficacy against numerous strains of microbes (30).

According to *Nisar et al.*, the phenolic compounds in turmeric such as curcuminoids have antibacterial activities against *E. coli* and *S. aureus* (31). However, *Gul* and *Jehan* showed that the different turmeric extracts such as aqueous, n-hexane, methanolic, and chloroform exhibited dissimilar antimicrobial activity when tested against the same microbe (e.g. *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*) (32). This is mainly due to the solvent and the extraction system that play an important role in the final antimicrobial activity of the extract based on what *Ríos* and *Recio* explained (33).

Based on the above, the number of cases (MDR and the effect of solvent on the extract efficiency), this study is designed to investigate the antimicrobial effects on pathogenic bacteria of gram-positive (*S. aureus*), gram-negative (*E .coli*) fungus (*C. albicans*) of the dry pure turmeric powder extracts in three different solvents (distilled water, pure ethanol, and pure ethyl acetate) by broth microdilution techniques.

All antimicrobial results of the current study agree with several previous studies. To illustrate, the results of our study regarding the aqueous extract of turmeric rhizome effects on *S. aureus* and *E. coli* are in line with their (16, 28, 30) results.

Based on the study of *Sharma* and colleagues, it was found the ethyl-acetate turmeric extract possessed considerable antibacterial activity against *S. aureus* this finding was consistent with this study's finding (34).

At the same time in this study, the current results indicated that the ethanol turmeric extract did not play any inhibitory effect against *E. coli,* as well as *Nanasombat* and *Lohasupthawee* published by (35,36). Howeverer, regarding *S. aureus* it is contrasted with the results published by *Saleh* and *colleagues* when they found that *Curcuma longa* ethanolic extracts had an antimicrobial effect against *S. aureus* with a 25 mm inhibition zone (35).

In the present study, the aqueous turmeric extract and the ethyl-acetate turmeric extract had the same inhibitory effects on the gram-positive bacteria. To illustrate, when 0.39 µg\µl of the aqueous turmeric extract inhibited the *S. aureus,* ethyl-acetate turmeric extract also needed 0.39 µg\µl to do the same effect (Figures 1&3).

Although only the aqueous turmeric extract had inhibitory effects on *S. aureus, E. coli,* and *C. albicans,* it was more effective against bacteria. To clarify, the concentration of the aqueous turmeric extract to play an antifungal effect was (MIC= 50 µg\µl), this concentration was diluted 7 times to reach 0.39 µg\µl which was the concentration of the aqueous turmeric extract to perform the same action that prevented *S. aureus,* and it was diluted 5 times to reach 1.562 µg\µl to inhibit *E. coli* growth (Figure 1).

Among all these tested samples of turmeric powder extracts (aqueous extract, ethanol extract, and ethyl acetate extract), the ethanol turmeric extract was not effective against *S. aureus* nor *E. coli* and *C. albicans* (Figure 2).

In the present study, the aqueous, ethanol, and ethyl-acetate extracts of turmeric exhibited different behaviors against *S. aureus*, *E. coli,* and *C.*

albicans, this was well detailed before (20) and explained that the antimicrobial properties of turmeric extracts different by using different solvents (20). Because the solvent of extraction plays a main role in adequate types of phytochemical compounds in the final extract (37).

On the other hand, many previous studies obtained irreconcilable current findings such as *Abbasi* found that the hot and cold water extracts of turmeric show no antibacterial activity at all against such as *E.coli* but the ethanolic extract possessed it (27). Additionally, many previous studies obtained negative results regarding the turmeric extract such as *Kebede* and *colleagues* reported that the dry pure turmeric extract did not have any inhibition activity on *E. coli* (38).

This can be explained by *Akrayi* observing that the antimicrobial effect of plant extract differs between plants in several studies carried out because every study carries out the plants in different areas of the world, thus many factors such as the vegetation cycle stage and structure of extracted product which play different activity against the same microbial strains (39).

Previously, researchers have explained the antimicrobial mechanism of several herbal spices. Primarily, the action of different compounds such as phenolic compounds with bacteria wall proteins includes dipole-dipole attraction and hydrophobic interaction which leads to the distribution of the cell membrane balance, then collapse of the cell wall and destructive of the electron transport chain (31). *Gul* and *Bakht* reported that the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol, and valeric acid in turmeric gives it antimicrobial properties (32). *Nisar* and colleagues showed that the antibacterial potential effects of aqueous turmeric extracts are probably because of the anionic elements like nitrate, and thiocyanate, this gives the turmeric aqueous extract the activities against many MDR-tested bacteria (31). The antibacterial activity of alcoholic turmeric extract may be due to its ability to dissolve the phenolic compounds from herbal like curcumin which is a powerful antimicrobial agent (4,40).

The different response between gram-positive bacteria and gram-negative bacteria to the same turmeric extract refers to the difference in the cell wall structure of these two bacteria strains (38). To illustrate, gram-negative bacteria like *E. coli* have a rigid lipopolysaccharide on an external membrane, thus limiting the penetration of hydrophobic compounds across it. In contrast, the Gram-positive bacteria's cell membrane has a thick peptidoglycan wall that is not solid enough to prevent the diffusion of small hydrophobic antimicrobial molecules (31,38).

Conclusion

To conclude, the development of in vitro trials has led to the detection of valuable effects of turmeric, this helps be close to reaching newer medical agents, especially antibiotic compounds. This study demonstrated the antimicrobial activities of the aqueous turmeric extract and the ethyl-acetate turmeric extract. Although it established that the aqueous turmeric extract and the ethyl-acetate turmeric extract had the same power effect on inhibition of the *Staphylococcus aureus* (*S.aureus*) growth, the ethyl-acetate turmeric had just activities against gram-positive bacteria (*Staphylococcus aureus*). On the other hand, only the aqueous turmeric extract inhibited the gram-negative bacteria (*Escherichia coli*) and the fungus *C. albicans*.

We need deeper analytical studies to look at the compounds responsible for the antimicrobial activities of aqueous turmeric extract and ethyl turmeric acetate extract in this study.

Authors contribution

Mohammad Al-Najjar: Conception, Design, Resources, Materials, Critical Review, Feras Darwish Elhajji: Conception, Design, Supervision, Resources, Critical Review, Ruaa R. Al-Alwany: Design, Analysis and interpretation, Saleh Al.Naji: Literature search, lab work, Zaid Al.Herbawi: Literature search, lab work, Sawsan Abu Jamma'ah: Supervision, Shatha Alshaer: Data collection and processing, Analysis and interpretation, Writing manuscript.

Declaration of Competing Interests:

The authors have no conflict of interest with anybody anywhere.

Data availability

Data will be available upon request.

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