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A Comparative Study Between the Antibacterial Effect of Alcoholic Extract and Hot Water Extract of Fresh Turmeric on Some Pathogenic Bacteria

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ABSTRACT

Background: *In a study evaluating antibiotic susceptibility, Staphylococcus aureus exhibited resistance to Penicillin and Clindamycin but was sensitive to Levofloxacin and Chloramphenicol. In contrast, Streptococcus pyogenes showed resistance to all four antibiotics. Pseudomonas aeruginosa demonstrated resistance to Amikacin, Levofloxacin, and Ceftazidime, while showing sensitivity to Meropenem and Imipenem, and resistance to Piperacillin/Tazobactam. Escherichia coli was highly sensitive to all antibiotics except Ceftazidime, which showed high resistance. Salmonella typhi exhibited resistance to Ceftazidime and Piperacillin/Tazobactam, intermediate sensitivity to Imipenem and Levofloxacin, and sensitivity to Meropenem and Amikacin. The study also assessed the inhibitory activity of turmeric extracts. The alcoholic extract of turmeric was highly effective against all tested bacteria compared to the hot water extract. At 200 µg/ml, both extracts inhibited all bacteria, with the least effect on Pseudomonas aeruginosa. At 150-100 µg/ml, both extracts inhibited all bacteria except Pseudomonas aeruginosa. At 50 µg/ml, the alcoholic extract showed no inhibitory activity against Pseudomonas aeruginosa and Staphylococcus aureus but inhibited other bacteria, while the hot water extract only inhibited Streptococcus pyogenes. These findings highlight the concentration-dependent antibacterial efficacy of turmeric extract*

Keywords: Alcoholic Extract, Hot Water Extract, and Fresh Turmeric.

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INTRODUCTION

The current synthetic drug-based therapeutic approach is costly and alters metabolism and genetics. However, to manage the onset and course of the condition, a safe and effective treatment method is required. In this sense, medicinal plants and their components are crucial for managing illnesses by modifying biological processes. (1). The bioactive ingredient that gives turmeric its many pharmacological properties, such as anticancer, antibacterial, anti-inflammatory, antioxidant, and antidiabetic effects, is curcumin, a yellow molecule that is extracted from the rhizome of Curcuma longa (2). Turmeric is grown primarily in tropical and subtropical locations, and India produces the majority of it. Historically, it has been used to flavor food, dye clothing, and heal numerous human diseases. (3). It has been demonstrated that curcumin possesses direct, broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria. (4).

Pseudomonas aeruginosa is a non-sporing, non-capsulated, gram-negative bacillus. It can affect almost any external site or organ.

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Infection in hospital includes urinary tract infection, infected ulcers, burns and eye infection (5). Pseudomonas aeruginosa is one of the leading causes of hospital-acquired and chronic infections, and it is associated with increased antibiotic resistance, morbidity, and mortality (6). Escherichia coli is a Gram-negative bacterium (aerobic) from Enterobacteriaceae family, rod-shaped, motile or non-motile. Cause of enteritis, urinary tract infection, septicaemia and other clinical infection (7). Antibiotic resistance in Escherichia coli is quickly increasing, particularly against fluoroquinolones and third- and fourth-generation cephalosporins. Surprisingly, the majority of these multidrug-resistant strains are acquired in the community rather than in medical settings (8-9). Salmonella typhi occasion bacterial infection called typhoid, characteristic of bacteria are gram-negative, rod in shape, and have flagellum that having capsule which affords bacterial virulence protecting it from phagocytosis (10).

The advent of multidrug resistance to routinely used antibiotics has worsened the treatment and management of enteric fever, which is acknowledged as one of the most difficult challenges in disease management (11) Staphylococcus aureus is a Gram-positive bacteria with a cocci-like form with clusters described as "grape-like." Skin diseases include abscesses, respiratory infections such sinusitis, and food poisoning (12). Streptococcus pyogenes, sometimes known as "group A streptococcus" (GAS), is a Gram-positive bacterial pathogen that can cause both non-invasive and invasive illness (iGAS), as well as nonsuppurative complications. This includes pharyngitis, scarlet fever, impetigo, cellulitis, type II necrotising fasciitis, streptococcal toxic shock syndrome, acute rheumatic fever, and post-streptococcal glomerulonephritis (13). GAS (group A streptococcus) generate and release a vast number of physiologically active extracellular products, including hyaluronidase and streptokinase, which are important virulent factors in the pathogenicity of S Streptococcus pyogenes (14).

The study was aimed to investigate the antibacterial properties of turmeric extract (either hot water or alcoholic extract) against a selection of pathogenic bacteria known to cause infections in humans, in order to assess their potential as natural antibacterial agents for health applications or food preservation

MATERIALS AND METHODS

Materials:

* **Fresh Turmeric Rhizomes:** Obtaining fresh turmeric rhizomes from the local market in Najaf City - Najaf Governorate – Iraq.

*__Bacterial isolates__: Bacterial isolates *Pseudomonas aeruginosa* and *Escherichia coli* and *Salmonella typhi* and *Staphylococcus aureus* and *Streptococcus pyogenes* Obtained From Post graduate students in The Department of Biology-College of Science - University of Kufa and confirmed By cultural and biochemical examination(**15-16**) and ultimately by VITEK-2 compact system.

* **Chemical and cultural material** as Ethanol alcohol and Distill water and MacConcky agar and Muller Hinton agar and Nutrient agar and Normal Saline and some Antibiotic discs use to gram positive bacteria as (Penicillin 10 unit – Levofloxacin 5 µg –Chloramphenicol 30 µg -Clindamycin 2 µg) and gram negative bacteria as(Amikacin 30 ug –Ceftazidime 30 ug - Meropenem 10 ug –Impenem 10 ug - Levofloxacin 5 ug -piperacillin /tazabactam 100/10) and Dimethyl Sulfoxide.

Methods:

1. **Preparation and extraction of turmeric**

The first step for extraction involved the preparation of dry powder from fresh turmeric

rhizomes. For this, fresh turmeric were brought from the local market in Najaf City - Najaf Governorate – Iraq. The turmeric were then thoroughly washed. After washing, they were peeled, sliced into pieces and then dried by an electric oven for 5 days to remove the moisture. Removing of sufficient moisture content of crude drugs was necessary to improve its quality and make it resistant to the growth of microorganisms. The dried turmeric samples were then powered by mechanical grinder and sieved to give powdery form. The powder was stored in polythene bag at room temperature before extraction.

A-Hot water extract of turmeric

Prepare 50 grams of dry turmeric powder and add to it 500 milliliters of boiling distilled water with continuous stirring. Leave it in a shaking incubator at a temperature of 35°C for 24 hours. Then filter first using several layers of medical gauze, followed by Whatman No. 1 filter paper. Take the filtrate and evaporate it using a rotary evaporator at a temperature not exceeding 40°C until a thick liquid is obtained. Then dry the liquid in an incubator at a temperature of 35-40°C for 2-3 days until a dry powder is formed. Collect the powder in clean and sterile glass bottles, and store it in a refrigerator at a temperature of 4°C, according to **(17)** with some modifications.

B- Ehanolic alcohol extract of turmeric

The same steps for preparing the hot water extract were followed, except 250 milliliters of 75% ethyl alcohol was used instead of distilled water according to **(18).** Then, a stock solution was prepared by taking 0. 4 mg, 0. 3 mg, and 0. 2 mg , 0. 1 of turmeric extract powder of both hot water and ethanoic alcohol to dissolve them in 2 ml of solvent to prepare concentrations (200-150 -100-50) ug/ml, respectively, and the solvent used was consist of Dimethyl Sulfoxide, (DMSO) then filter the storage solution for sterilization with special filters Whatman membrane filter (0.22) µm according to**(19).**

2. Inoculum preparation

The method mentioned by (**20**) was used to prepare the bacterial inoculum by growing the activated bacteria on Nutrient Agar medium and incubating it at 37 degrees for 24 hours, then transferring ten colonies of each type of bacteria used in the experiment and under sterilization conditions to a test tube containing on 5 ml of Nutrient Broth and then incubated at 37 °C for (4-6) hours

1. Antibiotic Susceptibility Test

For this test, the fresh inoculums of test microorganism were taken. five sterile petri plates were taken and Mueller Hinton agar was poured to it and allowed to solidify at room temperature. Sterile Mueller Hinton agar plate was uni-formly swabbed with a specific bacterial culture. Then, four different antibiotics discs namely Penicillin ,Levofloxacin,Chloramphenicol ,Clindamycin having respective concentrations of 10µg, 5µg , 30µg and 2µg were placed in plate inoculated with Gram-positve bacteria and another six different antibiotics discs namely Amikacin ,Ceftazidime , Meropenem ,Impenem, Levofloxacin 5 and piperacillin /tazabactam having respective concentrations of 30µg, 30µg , 10µg, 10µg, 5µg and 100/10 µg were placed in plate inoculated with Gram-negative bacteria. The plates were incubated at 37oC for 24 hours (**20).**

2. Study the effect of hot water and alcoholic extract of turmeric on the growth and activity of bacterial species and the comparison between them

Minimum inhibitory concentration by using Agar well diffusion method was used to observe the effect of the extracts on the growth of the isolated bacteria under study. Spread 0.1 ml of the bacterial inoculums on the surface of

The Muller Hinton agar then the dishes were left on the medium at room temperature for 15 minutes to allow for absorbing the inoculum, then work 5 wells with 10 mm diameter by cork borer and then added 100 µl of each concentration of the plant extracts under study in 4 well, and At the same time 100 µl of sterile distil water was placed in last well as control instead of the plant extract by using micropipette, and the addition must be on the surface of the culture media carefully, then the petri dishes were incubated in 37°C for 24hr. **(21).**

RESULTS AND DISCUSSION

In the study of antibiotic Susceptibility Test of four different types of antibiotics on Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*, the results appeared clearly *Staphylococcus aureus* have resistance against Penicillin and Clindamycin while have sensitive against Levofloxacin and Chloramphenicol while *Streptococcus pyogenes* have resistance against all antibiotics Penicillin , Clindamycin, Levofloxacin and Chloramphenicol this results can showed clearly in Table (1) below . On another hand antibiotic susceptibility test of six different types of antibiotics on Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* the results appeared clearly *Pseudomonas aeruginosa* have resistance against Ceftazidime , Amikacin and Levofloxacin while have sensitive against Meropenem ,Impenem and piperacillin /tazabactam while *Escherichia coli* have sensitive against most antibiotics except Ceftazidime have resistance against it on another hand *Salmonella typhi* have resistance against Ceftazidime and piperacillin /tazabactam while have sensitive against Meropenem and Amikacin and have intermediate against Impenem and Levofloxacin this results can showed clearly in Table (2) below:

Table: (1) **The inhibition zones of standard antibiotics disc against *Staphylococcus aureus* and *Streptococcus pyogenes***

Bacteria	**Diameter of inhibition zone for antibiotics disc (mm)**			
	Penicillin	Clindamycin	Levofloxacin	Chloramphenicol
Staphylococcus aureus	**0**	**0**	**27**	**20**
Streptococcus pyogenes	**9**	**0**	**9**	**8**

According to Table (1) that refer to the results of antibiotic Susceptibility Test of *Staphylococcus aureus* near to the results of study recorded by (**22**) .The results showed resistance to Clindamycin and sensitive to Levofloxacin .The results agree with another study recorded by **(23)** that showed resistance to penicillin and sensitive to Levofloxacin and Chloramphenicol. On another hand According to Table (1) that refer to the results of antibiotic susceptibility test of *Streptococcus pyogenes* that disagree with study introduced by **(24).** showed sensitive to Levofloxacin, penicillin and Chloramphenicol and resistance to Clindamycin . another study show completely disagree with study introduced by **(25).** showed sensitive to Levofloxacin, penicillin ,Chloramphenicol and Clindamycin .the cause of resistance may be existence enzymes or resistance genes.

Table: (2) The inhibition zones of standard antibiotics disc against *Pseudomonas aeruginosa* , *Escherichia coli,* and *Salmonella typhi*.

Bacteria	Diameter of inhibition zone for antibiotics disc (mm)					
	Meropenem	Imipenem	Levofloxacin	Ceftazidime	Amikacin	piperacillin /tazabactam
Pseudomonas aeruginosa	**23**	**24**	**12**	**00**	**9**	**26**
Escherichia coli	**33**	**30**	**25**	**13**	**22**	**23**
Salmonella typhi	**24**	**22**	**22**	**9**	**30**	**17**

According to table (2) that refer to the results of antibiotic Susceptibility Test of *Pseudomonas aeruginosa* near to the results of study recorded by (**26**).The results showed resistance to Amikacin , Levofloxacin, ce and sensitive Meropenem and Imipenem except piperacillin /tazabactams showed resistance to it while disagree with to the results of study recorded by (**27-28**) that showed sensitive to all antibiotics . The results shown in the table (2) for *Escherichia coli* are similar to the results recorded by (**29**) which indicate a high sensitivity of the bacteria to all antibiotics used except Ceftazidime have high resistance to it. On another hand *Salmonella typhi* have resistance aganist Ceftazidime and piperacillin /tazabactams and intermediate to Imipenem and Levofloxacin and sensitive to Meropenem and Amikacin that disagree with study introduced by **(30).** That showed sensitive to all antibiotic .

Table : (3) The inhibition zones of of alcoholic and hot water extract of turmeric against *Pseudomonas aeruginosa* ,*Escherichia coli, Salmonella typhi* ,*Staphylococcus aureus* and *Streptococcus pyogenes*.

Bacteria	Diameter of inhibition zone for each concentration (mm)								
	Alcoholic				Hot water				
	200 ug/ml	150 ug/ml	100 ug/ml	50 ug/ml	200 ug/ml	150 ug/ml	100 ug/ml	50 ug/ml	Control
Pseudomonas aeruginosa	**13**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**
Escherichia coli	**19**	**16**	**13**	**12**	**17**	**15**	**11**	**0**	**0**
Salmonella typhi	**20**	**17**	**14**	**11**	**18**	**13**	**12**	**0**	**0**
Staphylococcus aureus	**19**	**15**	**13**	**0**	**16**	**14**	**11**	**0**	**0**
Streptococcus pyogenes	**20**	**17**	**15**	**13**	**18**	**15**	**12**	**11**	**0**

In the study of the effect of agar well diffusion plate assay method, the results appeared clearly the effect the alcoholic extract of turmeric was highly effective against all bacteria used in the study compared with hot water extract of turmeric that have less effect , this results can showed clearly in table (3) and figure (1). This result compatible with study introduced by **(31).**

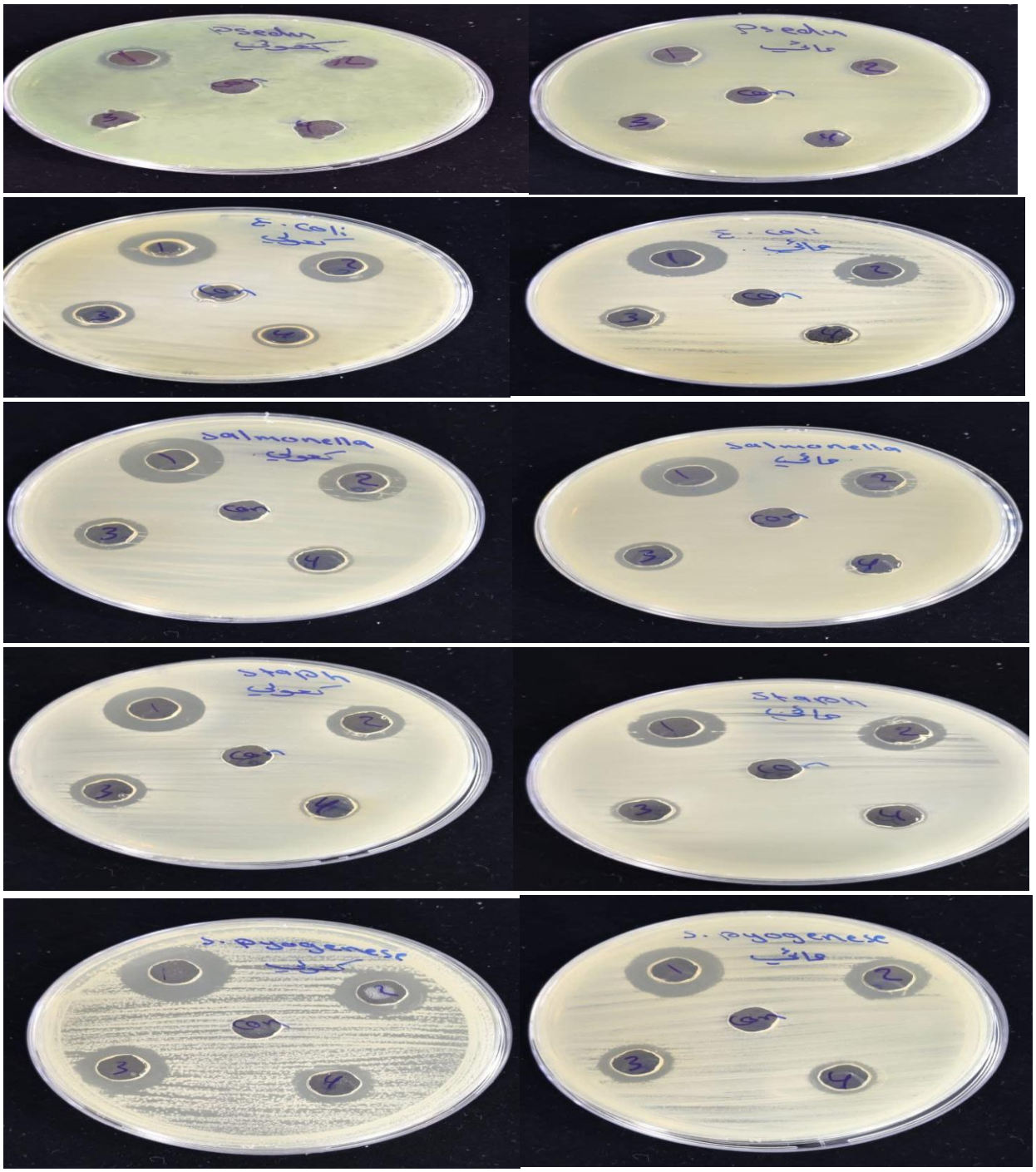

Figure: (1) The antibacterial effect of alcoholic and hot water extract of turmeric against *Pseudomonas aeruginosa,Escherichia coli Salmonella typhi ,Staphylococcus aureus* and *Streptococcus pyogenes*. by agar well diffusion method (10 mm).

At a concentration 200 ug/ml of alcoholic and hot water extracts of turmeric were found to have inhibitory activity against all types of bacteria used in the study, but showed the least inhibitory activity against *Pseudomonas aeruginosa* on another hand at a concentration150-100 ug/ml of alcoholic and hot water extracts of turmeric were found to have inhibitory activity against all types of bacteria used in the study, but showed no inhibitory activity against *Pseudomonas aeruginosa* while At a concentration 50 ug/ml of alcoholic extract of turmeric show no inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and have inhibitory activity another bacteria on another side the concentration 50 ug/ml of hot water extract of turmeric show no inhibitory activity against all types of bacteria except for *Streptococcus pyogenes*, where hot water extract at this concentration exhibits inhibitory activity. This result disagree with the study recorded by **(31-32).** I could not find a study that matches the results I obtained. The strong effect of the turmeric extract on bacteria may be attributed to the use of fresh turmeric rhizomes instead of the commercially available turmeric powder

CONCLUSIONS

My study shows that turmeric contains potential antibacterial compounds that could be valuable for developing new medications, presenting a promising therapeutic option for the pharmaceutical. It also revealed that the alcoholic extract of turmeric is more effective against pathogenic bacteria compared to the hot water extract, although the aqueous extract of turmeric still has a relatively strong effect

It has been suggested for use in the production of ointments and medicines due to its high effectiveness against bacteria that infect the skin, in addition to its use in the production of natural antibiotics.

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