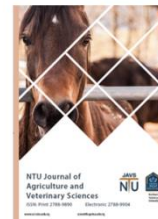




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Identification of the active compounds and estimation of the amount of curcumin in the hot aqueous extract of *Curcuma longa* roots and evaluation of its inhibitory effectiveness against microorganisms

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ABSTRACT

This study was conducted to detect the bioactive compounds in hot aqueous Turmeric (*Curcuma longa*) roots extract and study its effectiveness in inhibiting a number of gram-negative and positive bacteria and two types of molds. It was found that the turmeric extract contains a number of active compounds, the most important of which are flavonoids and resins, in addition to volatile oils, while it does not contain The extract contains alkaloids, glycosides, saponins, steroids, tannins, terpenes, and coumarins, and the total amount of phenolic substances in the extract amounted to 7.20 mg g⁻¹ of gallic acid, and the percentage of curcumin in the extract amounted to 8.49 mg g⁻¹. Turmeric root extract did not show high inhibitory activity at all concentrations against gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumonia* bacteria. On the contrary, gram-positive bacteria had weak resistance to the extract, specifically at concentrations of 150 and 200 mg g⁻¹, as they reached diameters of *Staphylococcus aureus* bacteria 14.00 and 14.50 mm at concentrations of 150 and 200 mg ml⁻¹, respectively, and *Bacillus subtilis* bacteria 22.50 and 24.50 mm at concentrations of 150 and 200 mg/ml, respectively. The results showed that there were significant differences in the percentage of mold inhibition, and the highest percentage of inhibition reached 85.24 for the turmeric root extract at a concentration of 200 mg ml⁻¹ for *Alternaria alternata* mold, and the lowest percentage of inhibition reached 28.57% at a concentration of 50 mg ml⁻¹ for *Aspergillus niger*.



Introduction

Turmeric (its scientific name is *Curcuma longa*) is one of the most important plants of the ginger family Zingiberaceae, whose cultivation and uses have been famous in India since ancient times, but now its cultivation has succeeded in many countries around the world, including China and Latin America. Turmeric is used in food manufacturing as a flavoring spice and as an agent. It is a coloring and preservative, in addition to having medical uses. It is used in the manufacture of cosmetics and textiles and is also considered an insect repellent [1].

Turmeric roots are the most widely used part of the plant because of the bioactive chemical compounds it contains, the most important of which are flavonoid phenolic compounds called curcuminoids, in addition to the presence of volatile oils [2].

The results of chemical analysis showed that turmeric contains 8.92% moisture, 2.85% ash, 9.42% protein, 4.60% fiber, and 6.85% fat. In addition, turmeric roots contain many bioactive compounds, such as phenols, flavonoids, tannins, glycosides, saponins, and sterols. The most important phenolic compound is the group of curcumin compounds, which constitute 1-6% of the total turmeric powder (dry weight) and include curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC), whose percentages are 77%, 17%, and 3-6%, respectively [3].

Curcumin is one of the phenolic compounds found in many plants, especially the turmeric plant, and it is one of the flavonoid anthocyanin pigments. Scientific research that has extended for more than four decades has confirmed that curcumin is a chemical preventive agent, a therapeutic agent against many chronic diseases, and an antioxidant and antimicrobial agent. Pathogenicity: The World Food and Drug Administration (FDA) indicated that curcuminoids are recognized and safe for health and determined an acceptable daily intake rate of 12 grams per day. The dose can be taken orally [3], [5], [6], [7].

Curcumin is an anti-inflammatory, anti-cancer, stimulant for the heart and blood vessels, and is beneficial for diabetics, in addition to being anti-bacterial, anti-fungal, and antioxidant, which makes it a preservative agent in foods ([8], [9], [5].

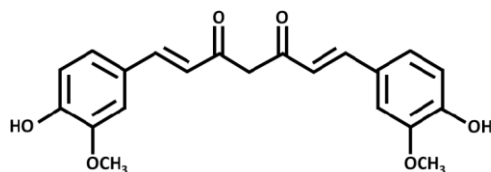


Figure 1. shows the chemical structure of curcumin [10]

Approximately 0.55 mg of curcumin can be extracted with cold water per gram of turmeric powder, and the amount of curcumin was 2.42 mg g⁻¹ turmeric for hot water extraction [24]. The aqueous extract of turmeric has proven its antimicrobial activity against many Gram-positive and Gram-negative bacteria, the most important of which are *staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* [11].

The methanolic extract of turmeric has proven its inhibitory effectiveness against many microorganisms, the most important of which are *Bacillus cereus*, *Pseudomonas* spp., *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Aspergillus* spp., and *Penicillium* spp., with a final concentration of 20 mg/mL. The zone of inhibition shown by the plant extracts against the tested organisms ranged. between 7.0 and 20.0 mm [3].

MATERIAL AND METHODS

These tests were conducted in the laboratories of the Food Science Department at the College of Agriculture and Forestry at the University of Mosul

Hot water extraction of turmeric roots

The method of Gulcin et al. [12] was used to obtain a hot aqueous extract of turmeric roots containing bioactive substances. The method is done by weighing 25 grams in a beaker containing 250 ml of distilled water, boiling it for 30 minutes on a magnetic hot plate stirrer, filtering it with gauze, filtering it with Whatman No. 1 filter paper, then concentrating the extract and drying it in a Proodit-95-10026 desiccator. Italian origin, for 8 hours at a temperature not exceeding 40°C to obtain powdered extracts.

Determination of the total content of phenols

The concentration of total phenols in the extract obtained in paragraphs (2-1) was measured using a phenol reagent according to the method mentioned by Laouini and Ouahrani [13]. The standard curve was prepared by taking different concentrations of gallic acid: 0, 20, 40, 60, and 80 mg ml⁻¹. As shown in Figure (2), to calculate the percentage of total phenols in aqueous turmeric extract, the amount of total phenolic substances is expressed as the equivalent of mg of gallic acid per gram of sample (dry weight), and total phenols are calculated according to the equation mentioned by Nurhasnawati et al. [14], which states the following:

$$TPC = (C \cdot V)/W$$

$$TPC = (CV)/W$$

TPC = total phenolic content

C = sample concentration taken from the calibration curve

V = volume of sample solution

W = weight of extract

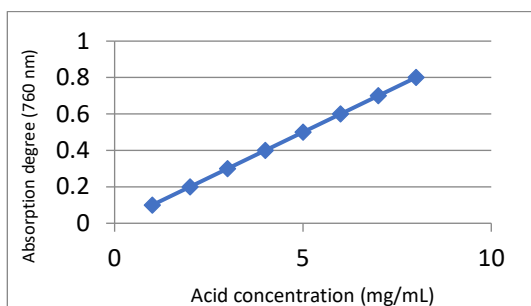


Figure 2. Standard curve for gallic acid. Calculation of total phenols for turmeric root extract

Chemical detection of the active chemical compounds of the extract. Flavonoids were detected according to the method mentioned by Auwal et al. [15] by adding 0.1 grams of zinc to the aqueous extract and 8 ml of concentrated sulfuric acid. If a red color appears, this indicates the presence of flavonoids. The method of Parekh and Chanda [16] was adopted to detect saponins and determine glycosides, while Ikpeama [3] method was used to detect alkaloids, and glycosides were estimated according to the method of Parekh and Chanda [16]. As for the detection of resins, it was done according to Mahmoud [17], with some modifications. According to the method, by adding 5 ml of ethyl alcohol to 0.25 g of extract powder, the mixture heated in a water bath until boiling for two minutes, then filter the solution and add to it 5 ml of distilled water acidified with HCL in case the turbidity in the solution is an indication of the presence of resinous materials and it is detected. Tannins were detected using the method [18], while the detection of terpenes and steroids was done using a method, and coumarins were detected by also following the method of [17] which stipulates adding a certain amount of the extract to a test tube and then covering it. The tube is covered with filter paper moistened with a diluted sodium hydroxide solution, then the tube is heated in a boiling water bath for a few minutes, and then the filter paper is exposed to ultraviolet rays. When the paper is colored yellow-green, this indicates the presence of coumarins. As for the detection of volatile oils, it was done by the method of Dahiru et al. [19] by adding 2 ml of diluted sodium hydroxide to the extract and a small amount of diluted hydrochloric acid. The solution was shaken well to see if white sediments formed, indicating the presence of volatile oils.

Measure the concentration of the active phenolic compound in the extract.

The concentration of the curcumin compound was diagnosed and measured using a German-made SYKMN HPLC high-performance liquid chromatograph with a separation column (18-ODS) with dimensions of 25 cm by 4.6 cm. the concentration of curcumin was diagnosed and measured according to the method mentioned by [20] with some modifications in the mobile phase

and concentrations. Using a mobile phase consisting of methanol and water (DDW) in a ratio of 50:50 and with a flow speed of 1.2 ml/min, The standard solution was prepared by dissolving 2 µg of curcumin in 1 ml of methanol and injecting 10 ml into the device. The compound was detected at a wavelength of 425 nm, while the sample prepared in paragraph (2-1) was detected under the same conditions used for the standard sample. Then the area of the standard compound was matched with the area of the compound present in the sample, and then calculations were performed to obtain the concentration.

Testing the effectiveness of the extract in inhibiting microorganisms

Testing the effectiveness of the extract in inhibiting Gram-positive and Gram-negative bacteria

This test was performed by the Agar Well Diffusion method mentioned by Manandhar et al., [21] by preparing the bacterial suspension by transferring individual colonies into Normal Saline solution and adjusting the concentration of the suspension using a standard (MacFarlad 0.5) to obtain a bacterial suspension containing a cell count of 1.5×10^8 cells ml^{-1} , prepare a Petri dishes containing Mueller-Hinton agar, contaminate the surface with the bacterial suspension using a sterile medical soap and spread the suspension evenly, drill holes in the medium contaminated with the bacterial suspension using a 6 mm diameter cork perforator for the purpose of injecting different concentrations of extracts. Prepare (50, 100, 150, and 200 mg) concentrations of turmeric root extract. Inject 100 milliliters of each concentration into a hole and leave the dish for half an hour to soak in the extract. Then transfer the dish to the incubator and incubate at 37°C for 24 hours. After 24 hours, the diameter of inhibition is measured in millimeters to determine the effectiveness of the extract and its concentrations against different types of bacterial isolates.

Testing the extract's effectiveness in inhibiting the growth of two mold

This test was conducted to determine the minimum concentrations of effective extracts inhibiting the growth of two types of molds, *Aspergillus niger* and *Alternaria alternata*, following the method of Dixit [23] , which is the Poisoned Food Technique, by preparing a solid nutrient medium of the PDA type, then pouring it into dishes in a size of 10 ml, adding 100 ml of extract (50, 100, 150, and 200 mg) till completely. After that, a disk of mold is grown, and the dish is incubated for 5-7 days at a temperature of 25°C. Before that, a the inhibition percentage was measured by the following:

$$\text{Percentage of inhibition} = \frac{A - B}{A} \times 100$$

A= Comparison Average Diameter, B= Average diameter of the sample colony

RESULTS AND DISCUSSION

Extraction

The aqueous extract of turmeric roots was obtained by following the steps mentioned in the working methods and converting part of the extract into a dried powder to be used to stabilize the required concentrations in later experiments.

Qualitative diagnosis of the active chemical compounds of the extract

Table 1 shows that the aqueous extract of turmeric roots contains many active compounds, the most important of which are flavonoids, resins, and tannins, in addition to volatile oils, while the extract does not contain alkaloids, saponins, glycosides, tannins, steroids, or coumarins. Some of the results of the turmeric root extract agreed with Ikpeama [3] especially in the presence of flavonoids, resins, and volatile oils, while the results differed in the absence of alkaloids, glycosides, and saponins.

Table 1. shows the active compounds in turmeric root extract

Extracts	Turmeric root extract
Flavonoids	+
Alkaloids	-
Saponics	-
Glycosides	-
Resins	+
Tannins	-
Terpenes	-
Steroids	-
coumarins	-
Volatile oils	+

+ : The result is positive, meaning that the compound is present

- : The result is negative, meaning that the compound is not present

Amount of total phenolic substances in the extract

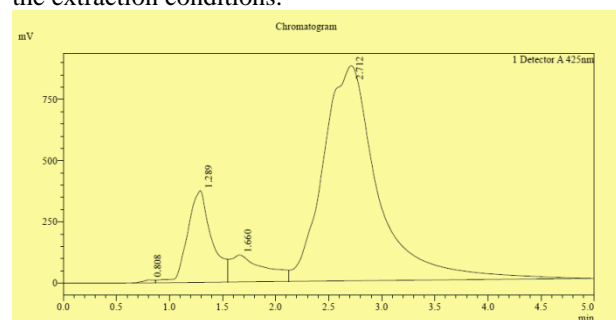
Plants are a rich source of phenolic compounds, which are biologically effective, especially because they are antimicrobial and antioxidant compounds that can provide oxygen atoms and suppress free radicals (Damodaren and Parkin, 2018). It is noted from Table 2 that the amount of total phenolic substances in the turmeric root extract amounted to 7.20 mg g⁻¹ gallic acid. The percentage of total phenolics in the turmeric roots was also close to what was mentioned by Tonfack et al. [25] who indicated that the percentage of total phenolics in the aqueous extract of turmeric reached 7.54. mg g⁻¹ gallic acid.

Table 2. shows the total content of phytolates in the aqueous extract of turmeric roots

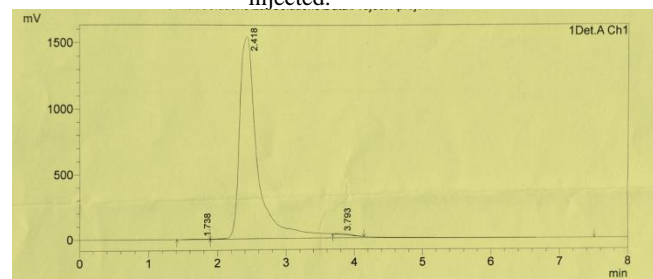
Extract	Total content of phenols (mg/g gallic acid)
Hot aqueous extract	7.20

The amount of active phenolic compounds in the extract

The amount of curcumin in the aqueous extract of turmeric roots was obtained using HPLC technology by obtaining curves that were compared with standard curves for standard compounds. Through curve (1) resulting from the aqueous extract of turmeric roots, the percentage of curcumin was determined, which is the most important phenolic compound in the roots. The percentage of curcumin reached 8.49 mg/g of turmeric from the extract when compared with the standard curve for curcumin (2), and this result differed from what was mentioned by Niamsa and Sittiwet [11], who indicated that the percentage of curcumin in turmeric reached 0.55 mg g⁻¹ upon extraction. cold water for turmeric, while the amount of curcumin was 2.42 mg g⁻¹ turmeric when extracted with hot water. This may be due to the Differences in genetic patterns of the turmeric type used and the source of its cultivation, in addition to the extraction conditions.



Curve 1. shows a diagram resulting from an HPLC device into which the aqueous extract of turmeric was injected.



Curve 2. shows a diagram resulting from an HPLC device in which curcumin (a standard substance) was injected.

Antibiotic activity of the extract on microorganisms

The extract effectiveness in inhibiting Gram-positive and Gram-negative bacteria

Table 3 shows that there are significant differences in the inhibitory activity of different concentrations of aqueous turmeric root extract on different types of Gram-negative and Gram-positive bacteria. The highest values of inhibition were for a concentration of 200 mg. There are significant differences in the inhibition of different types of bacteria, and the gram-positive bacteria were The less resistance there is to the extract, the higher the concentration. In contrast to the negative bacteria that gave the highest degree of resistance to the extract, turmeric root

extract did not give high effectiveness at all concentrations against Gram-negative bacteria, in particular *Escherichia coli* and *Klebsiella pneumonia* bacteria. While the positive bacteria were For gram dye, which has weak resistance to the extract, specifically at concentrations of 150 and 200 mg, as the diameters of *Staphylococcus aureus* bacteria reached 14.00 and 14.50 mm respectively , and *Bacillus subtilis* bacteria reached 22.50 and 24.50 mm respectively in the same concentrations. The inhibition results were similar with Niamsa and Sittiwet [11] in many types of bacteria, especially *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*, while the results differed with Hussein [22], who stated that the aqueous extract of turmeric gave inhibitory activity against many gram-negative bacteria but was not effective against gram-positive bacteria.

Table 3. Effect of different aqueous turmeric root extract concentrations on inhibition diameters of Gram-negative and Gram-positive bacteria

Damping zone diameter (mm)				
concentrations Types of bacteria	50	100	150	200
<i>Pseudomonas fluorescens</i>	6.50	7.00	8.50	10.00
<i>Pseudomonas aeruginosa</i>	7.00	7.50	8.50	9.50
<i>Escherichia coli</i>	6.50	7.50	8.00	8.50
<i>Klebsiella pneumoniae</i>	6.50	7.00	8.00	8.50
<i>Proteus mirabilis</i>	7.00	7.50	8.50	9.50
<i>Enterobacter aerogenes</i>	7.00	8.00	9.50	11.00
<i>Staphylococcus aureus</i>	7.00	9.50	14.00	14.00
<i>Bacillus subtilis</i>	19.00	20.50	22.50	24.50

*Different letters indicate significant differences at the 0.05 probability level.

*Figures are the average of three replicates.

The antifungal activity of the extract in inhibiting the growth of two types of mold

Table 4 shows the effectiveness of different concentrations of the aqueous extract of turmeric roots in inhibiting the growth of *Aspergillus niger* and *Alternaria alternata* molds. The results showed significant differences in the inhibition percentage when the concentrations and type of mold differed. The highest inhibition was 85.24 for the turmeric root extract at a concentration of 200 mg ml⁻¹ on the mold *Alternaria alternata*, and the lowest percentage of inhibition was 28.57%. At the concentration of 50 mg/ml on the mold *Aspergillus niger*, the turmeric extract gave varying results in terms of the percentage of inhibition against the mold *Aspergillus niger*, which amounted to 28.57.35% and 48.21. %, 60.71%, and 64.28% at concentrations of 50, 100, 150, and 200 mg ml⁻¹ respectively, while the inhibition percentages for *Alternaria alternata* mold reached 42.62%, 50.81%, 62.29%, and 80.32% at concentrations of 50, 100,

150, and 200 mg ml⁻¹. In a row, Al-Saidi [26] proved that some plant extracts had inhibitory activity against *Aspergillus niger* and *Alternaria alternata* molds.

Table 4. Effect of different aqueous turmeric root extract concentrations on growth inhibiting of *Aspergillus niger* and *Alternaria alternata* molds.

Transactions	Concentrations mg/ml	molds	
		<i>Aspergillus niger</i>	<i>Alternaria alternata</i>
		Percentage of inhibition	Percentage of inhibition
Turmeric root extract	50	28.57 f	42.62 e
	100	48.21 d	50.81 d
	150	60.71 c	62.29 b c
	200	64.28 b	80.32 a

*Different letters indicate significant differences at the 0.05 probability level

*Figures are the average of three replicates

CONCLUSION

It was found that the aqueous extract of turmeric roots contained active compounds, the most important of which was curcumin, which is a phenolic compound. The extract gave good results in inhibiting many bacteria and molds.

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Competing Interests

There are no competing interests.

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