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Inhibitory activity of curcumin extract against some bacteria causing food poisoning isolated from some ready-to-eat meals

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ABSTRACT

The current study aimed to extract curcumin from the rhizomes of turmeric *Curcuma longa* after exposing it to sonication, to confirm its specificity to curcumin through colorimetric tests, and Fourier Transform Infrared Spectroscopy (FT-IR), which proved it possesses the functional groups of curcumin. The efficiency of curcumin in its inhibitory activity against some gram-negative and gram-positive bacterial species isolated from some types of ready-to-eat foods randomly from fast food restaurants spread in Baghdad City, Iraq and with three activated colonies for each of them using the Well diffusion method.

Keywords: Curcumin extract, Pathogenic bacterial strains, Fast food restaurants.
Article type: Research Article.

INTRODUCTION

Recently, attention has turned to the plant kingdom as natural alternatives due to its abundance in nature, its cheapness, ease of use, and the fact that many of them contain effective substances, as well as the absence of dangerous substances (Naser Al-Isawi 2022; Salih *et al.* 2022; Al-Shurait & Al-Ali 2022) including curcumin, which is generally a polyphenolic phyto-constituent compound and one of the active substances. It is multifunctional and extracted either from the roots of the turmeric plant, *Curcuma longa*, or it can be prepared in the laboratory. It is characterized by its low molecular weight and fat-soluble, in addition to being a natural yellow dye. Its chemical composition is methane diferuloyl with the formula of $C_{14}H_{14}O_4$ and its scientific name is (1E, 6E)-1 7-bis-(4-hydroxy-3-methoxyphenyl)- hepta-1,6-diene-3,5-dione (Fig. 1A). Its other advantages includes changing its colours with different pH values, which can be considered as an indicator. On the pH, the reddish-brown colour represents the basicity, while it gives the yellow colour when it is in the neutral and acidic state (Fig. 2B). Recent studies have shown the possibility of using curcumin and anthony dye and cyanine as pH-sensitive indicators of fish freshness (Orteca *et al.* 2019; Zheng *et al.* 2020).

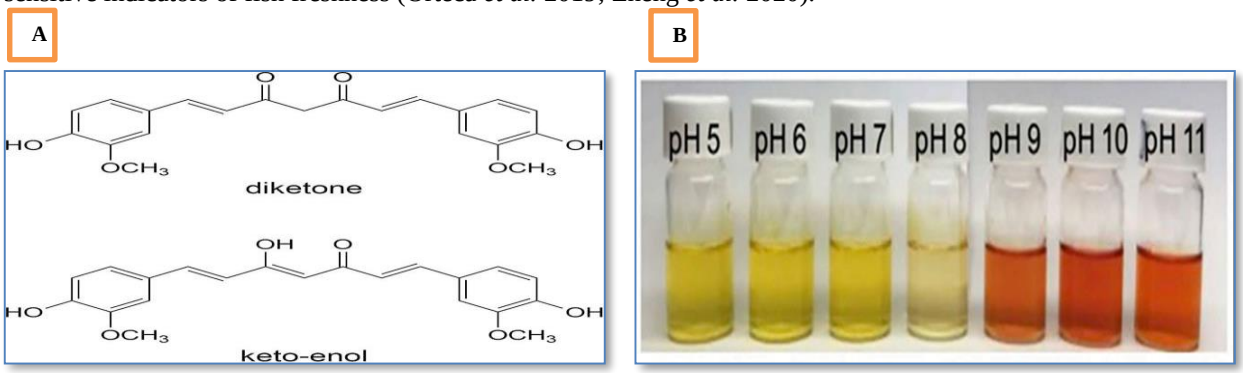

Fig. 1A: Chemical structure of curcumin; **B:** Colour of curcumin changes at different pH values (Zheng *et al.* 2020).

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There are three main components of curcumin isolated from the turmeric plant, including curcumin I, curcumin II, and curcumin III with 94, 5.7 and 0.3% respectively, which are responsible for giving turmeric yellow colour (Salehi *et al.* 2018) and this natural polyphenol is characterized by attracting interest. It is significant because of its non-toxicity to humans even at high doses (12 g day^{-1}; Shakeri & Sahebkar 2016). There were no toxic effects of curcumin when it was taken orally for three months at a dose of 8000 mg day^{-1}; Izui *et al.* 2016), while according to Niu *et al.* (2012), the curcumin that was taken orally, is excreted with faeces and urine after a rapid metabolism process to form many reduced products in the intestine. Most importantly, curcumin has an antioxidant, anti-tumor and anti-inflammatory effects as found by several authors when using at a dose of 180 to 200 mg kg^{-1} without any side effects (Sahebkar *et al.* 2016). In addition to its broad spectrum as an antibacterial, it exhibited suitable efficacy in the treatment of Alzheimer's disease, as its effectiveness is due to its possession of several functional groups, including the aromatic ring, which is a polyphenol linked to alpha and beta unsaturated carbonyl groups (He *et al.* 2015; Shakeri *et al.* 2019). After studying the natural elements that encourage self-suicide of malignant cells and developing them as a new generation of cancer drugs, the authors concluded that curcumin has distinct health properties and is the most effective, since it has a unique ability to shrink cells, break genetic material DNA, and impede the programming of signals (Piwocka *et al.* 2001). It was proven to have an inhibitory effect on the carcinogenic phIp during *in vivo* experiments. Despite its beneficial effects on human health, its hydrophobicity and instability both within *in vivo* and *in vitro* environments are limited. Its low biocompatibility has greatly limited its nutritional and medicinal applications (Piwocka *et al.* 2001). However, recent studies have indicated the possibility of its benefit in preparing medicinal compositions to treat bacterial infections instead of antibiotics, to which many pathogenic microorganisms have now become resistant as a result of random and unstudied uses. In this study, we have exhibited the effect of curcumin extract on inhibiting some bacterial strains isolated from fast food restaurants, which were described by (Karen 2016) as meals cooked outside the home. These foods contain high levels of saturated fats, carbohydrates, table salt and calories, with a low content of vegetables and fruits. The efficacy of natural curcumin extract will be presented against these isolated bacterial strains.

MATERIALS AND METHODS

Sample collection

In this study, we collected samples of some types of ready-to-eat foods from the fast food restaurants spread in Baghdad City, including meat and chicken shawarma, meat and chicken burger, meat and chicken pizza, meat and chicken kabab, flafel and appetizers to investigate some pathogenic microorganisms that cause food poisoning. These samples were placed in sterile polyethylene bags and closed tightly, then placed in containers with tight lids and transferred to the laboratory for microbiological examinations upon arrival. The tests were carried out to suit the microbes under consideration by weighing 10 g of food samples, each separately under sterile conditions, into the envelope of the Stomacher mixer. After adding an appropriate amount of physiological saline (0.85%) in glass bottles at a rate of 90 mL, and mixing the sample at a speed of 2000 cycles per minute for two minutes, the mixture was added to the rest of the contents of the sterile vial containing the physiological solution (0.85) % to obtain a dilution of 10^{-1}. Afterward, the dilutions were made up to 10^{-6}, then the culture was carried out by the method of decanting with three replications for each test using Nutrient Agar culture media and MacConkey Agar and Staphylococcus 110 with respect to the possibility of the presence of *Salmonella* bacteria. These bacteria, which is likely to be present in the samples, were activated by growing in the medium of Tetrathionate Broth, by weighing 10 g of food, and placing it in a 200-mL conical flask containing 90 mL of the medium. The samples were incubated at 37 °C for 24 hours, followed by transferring 1 mL by a sterile 1-mL pipette to a sterile Petri dish, pouring the culture medium *Salmonella* and *Shigella* agar and leaving the plates to incubate upside down at 37 °C for a period of 48 hours (APHA 1978) until the medium solidified.

Curcumin

1. Samples

An appropriate amount of turmeric rhizomes *Curcuma longa* were prepared from local markets and then transferred to the laboratory after cleaning them from impurities and dust. The quantity was crushed to obtain turmeric powder, which was placed in a clean and sterile container away from light and heat until use (Popuri 2013).

2. Extraction

Curcumin was extracted using an Ultrasound power sonic 405 device supplied by Hwashin Company of Korea. The process was performed according to the methodology described before Patil *et al.* (2018). After weighing 20 g of turmeric

powder and mixing it with 30 mL ethanol, then subjecting it to ultrasound for sonication for a period of time (15 min), we filtered the extracted solution to remove undissolved substances and stored at refrigerator temperature until use.

3. Curcumin diagnosis

The method of action described by Popuri (2013) was followed to verify the effectiveness of the curcumin extract by the following tests: Mixing 2 mL sulfuric acid with 2 mL curcumin appeared red, while the other tests included adding 2-3 mL hydrochloric acid on a filter paper after adding curcumin, followed by adding 2-3 drops of boric acid and drying it would appear red (similar to the colour of cherry red), while after adding 2-3 drops of ammonia solution, the colour changes to blue. The curcumin extract was also subjected to a diagnosis using the FT-IR technique, which was prepared by Shimadzu the Japanese Company of origin to find out the effective functional groups. The result was obtained in the form of a graph in which the X-axis represents the wavelength of the wave number, while the Y-axis represents the transmittance percentage.

Examining the inhibitory activity of curcumin against some pathogenic bacteria

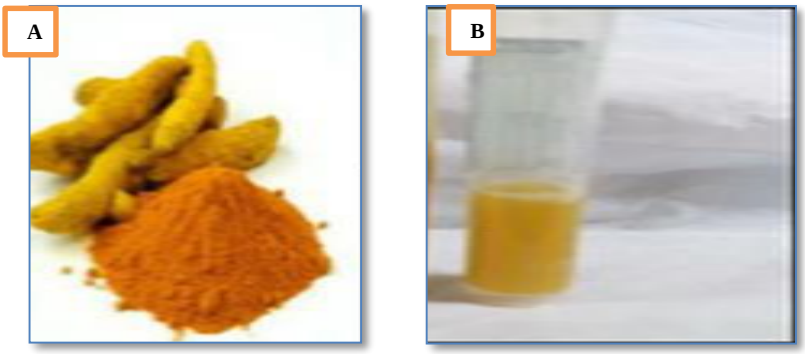
The Well diffusion method was used to detect the inhibitory activity of curcumin extract against the isolated bacteria. We spread 0.1 mL of the activated pathogenic bacterial isolates with three colonies each after a series of dilutions (0.25, 0.5 and 1) according to the original concentration of MacFarland tube (1.5×10^8 cells mL^{-1}) and spread on sterile Muller-Hinton agar medium, then poured into sterile L-shape Petri dishes. Pits were made with each bacterial concentration and for all test strains, as well as the curcumin-free positive control treatment. The dishes were incubated at 37 °C for 24 h for the pathogenic bacterial isolates. The diameter of the inhibition zone around each hole was measured using a graded ruler. The diameter of the areola surrounding the hole and free of growth represented the clear zone as the positive result of the test (Atlas 1995).

RESULTS AND DISCUSSION

The results of microbial culture of the samples taken, amounting to 60 from some fast food restaurants, showed the appearance of *Escherichia coli* bacteria, with a percentage of 41.66%, which is consistent with Bautista (2013) who isolated this bacteria from the appetizers with a percentage of 45.4%. Evidence of not cooking well is the ripening of the product, and they pointed out that despite being a natural flora in the intestines, it can cause various infections in many cases. The results of diagnostic tests showed the presence of *Salmonella* spp. at a rate of 8.3%. This is contrary to what was referred to by the Iraqi standard and international standards to the fact that foods are free of these pathogenic bacteria that cause many diseases for humans and animals alike. It may be due to the consumer or the possibility of contamination from the hands of the workers, as well as the failure to follow health and hygiene methods when preparing food. The presence of *Bacillus cereus* was monitored at a rate of 6%, especially in food appetizers that were not heat-treated, and this percentage can be explained by the fact that this bacterium is a soil microflora and therefore it came from raw materials (cabbage, carrots, mushrooms, eggplant etc.). Hence, it can be considered as plant-based ingredients, since they are carriers of these bacteria and their spores, which were not affected by washing or disinfection treatments with solutions, in addition to the lack of good preservation conditions. The coolant of these appetizers in the fast food restaurants during their preparation and presentation makes them vulnerable to contamination with this bacterium and thus pose a potential danger to the health of consumers, especially when this bacterium reaches to levels that make it cause poisoning. The results of microbial culture on the Staphylococcus 110 medium showed the presence of gram-positive *Staphylococcus aureus* at a rate of 16%. Its presence can be explained by the contamination of these meals that need either from the hands of workers or food processors in these restaurants, as well as leaving the food for a long period at an inappropriate temperature. It leads to an increase in its number beyond the permissible limit. In addition, poor attention to personal hygiene and the failure to implement effective control systems on these foods that are necessary to protect the health of local consumers and ensure their safety is one of the reasons for its existence.

Curcumin diagnosis

The results of the preliminary tests of the curcumin extract showed its dependence on curcumin in particular, through its positivity for the colours of the tests mentioned by Popuri (2013), who described the curcumin crystals powder with its yellow colour (Figs. 2A and B), in addition to its insolubility in water.



Figs. 3A: Tubers and turmeric powder; **B:** curcumin extract

FT-IR curcumin extract diagnosis

This test was conducted with the aim of diagnosing the active groups in the chemical composition possessed by curcumin extract within the frequency range between 400 and 4000 cm^{-1}. Notably, infrared waves are longer than visible or visible waves and shorter than radio waves. The results of this technique (Table 1 and Fig. 3) showed the presence of the stretched frequency group OH at wavelength 3674.14 cm^{-1}, while we noticed the presence of a band at length 1629.74 cm^{-1} due to the aromatic C=C group, while at wavelength 1415.65 cm^{-1} is due to the stretch frequency of the aliphatic CH group. A very strong band appeared at wavelength 1577.66 cm^{-1} representing the benzene ring, while a band appeared at wavelength 1510.16 cm^{-1} representing groups C=O and C=C. In addition, the aromatic C-O group was appeared at wavelength 1282.57 cm^{-1}, while we found the C-O-C group at wavelength 1026.06 cm^{-1}. These results are in agreement with those of Chen *et al.* (2015).

Table 1. Functional aggregates of curcumin extract using Ultrasound device.

Functional group	Absorption ranges (cm^{-1})
- OH stretch vibration	3674.14
aromatic moiety C=C stretching	1629.74
benzene ring stretching vibrations	1577.66
C=O and C=C vibrations	1510.16
olefinic C-H bending vibrations	1415.65
aromatic C–O stretching vibrations	1282.57
C–O–C stretching vibrations	1026.06

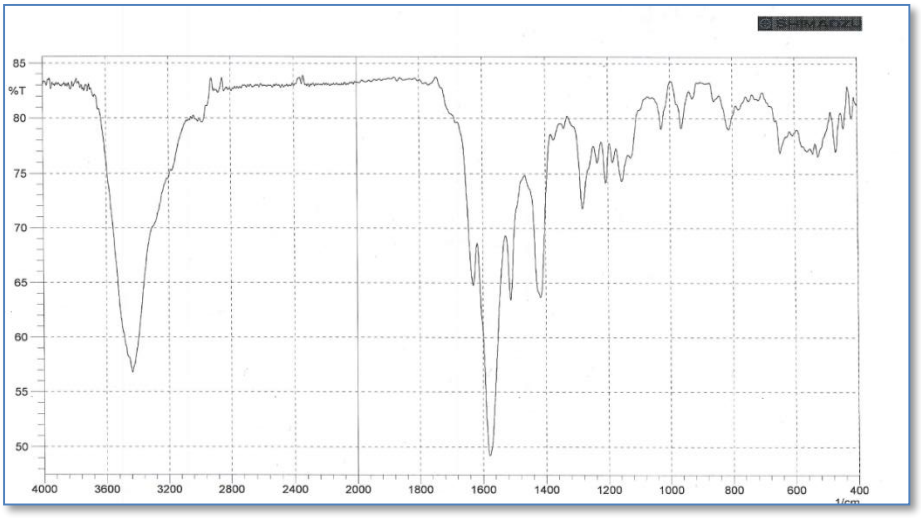


Fig. 3. FTIR spectra of curcumin extract using ultrasound device.

Inhibitory activity of curcumin against some pathogenic bacteria

Several studies indicated that curcumin has broad-spectrum antibacterial activity and strong biological activity against Gram-positive and Gram-negative bacteria (Khan *et al.* 2019). It was performed *in vitro* through its inhibitory activity against gram-positive and gram-negative pathogenic bacteria using the Well Diffusion method after isolating them from fast food restaurants and diagnosing them to ensure their return. For both of them, the results also showed that there is a discrepancy in the inhibition of these isolates depending on the concentrations of bacteria attenuated (0.25, 0.5 and 1; Table 2).

Table 2. Inhibitory activity of curcumin extract against some bacterial species isolated from the fast food restaurants.

Inhibition halo diameter (mm)				
Dilution Series	**Gram-negative bacteria**		**Gram-positive bacteria**	
	Escherichia coli*	***Salmonella* spp.***	***Bacillus cereus****	***Staphylococcus aureus****
0.25	16.5	9	12	17.5
0.5	21	11	18	16
1	23	14	22	20.5

Note***:** Average of three colonies per bacterium.

The difference in the degree of response and effect of the test isolates with curcumin can be attributed to the difference in the composition of the cell wall of these organisms and sometimes to the presence of genetic mutations in them (Zheng *et al.* 2020). The presence of several mechanisms through which curcumin can prevent the growth of bacteria either by targeting the bacterial cell membrane and cell wall curcumin, protein, DNA and other cellular structures or by inhibiting bacterial growth through the Quorum sensing (QS) system. Curcumin was also found to be a photosensitizer with phototoxicity and exerts bactericidal effects on various bacteria under blue light excitation. Furthermore, curcumin can have synergistic effects with other bacteriostatic substances in combination therapies to increase antibacterial properties.

CONCLUSION

The results of the current study showed the contamination of some fast food ready-to-eat bacteria with pathogenic bacteria, which could pose a potential danger to consumers' health, especially when their numbers reached levels that make them poisonous. Ultrasound is specifically to curcumin, and the results showed the efficiency of curcumin in its inhibitory activity against some bacterial isolates under test, and thus the possibility of applying it at the industrial level and adding it as an anti-microbial substance instead of chemical preservatives during the food manufacturing process.

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