

## Research Article

## CHEMICAL EXTRACTION OF CAPSAICIN FROM RED HOT IRAQI PEPPER AND STUDY OF THE ANTIMICROBIAL EFFECT OF ITS ALCOHOLIC EXTRACT

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## Abstract

Red hot Iraqi peppers were collected from Iraqi markets to estimate and extract capsaicin, which was done in the College of Health and Medical Technologies/Baghdad laboratory. Thin layer chromatography (TLC) technique was utilized to improve the presence of Capsaicin in the collected Chili. TLC confirmed the presence of Capsaicin in the plant extract as a major component compared with the standard Capsaicin in mobile phases: Toluene: Ethanol in different percentages as follows (7:3), (8:2) and (9:1). The current study involved the inhibitory efficacy of alcoholic red hot Iraqi pepper extracted on both gram-positive (G+) bacteria *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*), while gram negative (G-) bacteria were tested on *Escherichia coli* (*E. coli*) and *Klebsiella spp* (*K. spp*) with *Candida albicans*. Results show that the highest inhibitory action was against *Staphylococcus epidermidis* with an average diameter zone of inhibition of 16.0 mm at the concentration of 500 mg/10 mL, while a moderate inhibitory action against *S. aureus* and *Candida albicans*.

**Keywords:** Red Hot Iraqi Pepper, thin layer chromatography (TLC), Capsaicin, antimicrobial effect.

## INTRODUCTION

Recently, traditional medicine systems have been based on the phyto constituents on plants in many countries that play an essential role in healthcare. [1] In 2020, around 36 million tons of red peppers and green chilies were produced worldwide as Capsaicin or as Pimenta fruits. A high percentage of producing production was recorded in China around 46% of the total [2]. While in India, hot pepper was considered an important agricultural crop because of the economic important and the nutritional value of the fruits. [3] The chili pepper could be spelled chile or chilli which considered as fruit-of-plants of genus: Capsicum which is members of the nightshade family of Solanaceae. [4] The pungency (spicy hot) test of chili peppers was applied topically to two compounds capsaicinoids (trans 8-Methyl- N-vanillyl-6-nonenamide) and dihydrocapsaicin, that are responsible for about 90% of total hot teasty. [5] The quantity of capsaicin is depends on the growing change conditions that in dried chilies, which is hydrated fresh pepper, were approximately 4–10 times higher than in fresh case. [6] Depending on a number of characteristics, including location and type, capsicum plants have many names. The hot pepper plant needs a warm and sunny place, suitable temperatures for peppers are: 18-24 degrees Celsius during the day, 9-12 degrees Celsius at night. [7] In Iraq and depending on the availability of these conditions, the best period to plant pepper seeds is from February to April in addition to the abundance of sandy soils, which are estimated to be 19% of the cultivable land, that spread in wide area of Iraq. [8]

Several researchers studies *in vitro* and *in vivo* were conducted in capsaicin that showed the growing evidence for efficacy of capsaicin and effectiveness as antimicrobial and antioxidant and cytotoxic potential with no side effects [9, 10]. In addition to the wide applications and according to the mentioned above authors of this paper noted the need to study the properties of the Iraqi red pepper, Figure 1 for the first time in literature. This work aims to extract capsaicin from Iraqi red hot pepper and study the antimicrobial effect of its alcoholic extract.

## MATERIALS AND METHODS

## Chemicals



Figure 1. Red Hot Iraqi Pepper

Sigma-Aldrich compounds (St. Louis, US) provided all of the commercial compounds utilised in this study. The solvents and silica gel 60 plates with concentration zones were obtained from Merck (Darmstadt, Germany). Standard materials, capsaicin, were purchased from Chem-Impex Int'l Inc. (Chicago, US).

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## Microorganism isolates

The tested two bacteria species G- bacteria species (*E. coli* and *K. spp.*) and G+ bacteria (*S. aureus* and *S. epidermidis*) and *candida albicans* were isolated from the microbiological laboratory in the College of Medicine /Al- Mustansiriyah University.

## Plant collection

Red hot Iraqi pepper were collected from the city of Baghdad at March 2024. The collected amount was washed twice with tap water to remove foreign objects. Cut into small pieces to facilitate and speed up the drying process at room temperature. The dried pepper underwent grinding in order to obtain the powdered components which were transferred in to a 500 ml beaker to continue for the next preparation steps. Figure 2. [11].

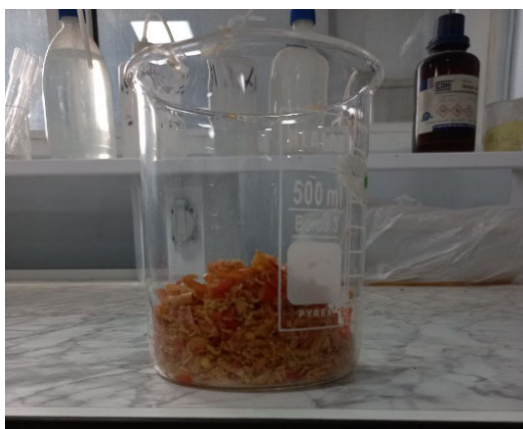


Figure 2. Dried red hot Iraqi pepper

## Extract preparation (Maceration method)

A 100 g of the dried pepper were added to 300 ml ethanol stirring by using a magnetic stirrer at room temperature for about a week and on the seventh day the extract was filtrated. The obtained filtrate was evaporated at room temperature; a brown color concentrated residue was obtained. [12] Figure 3.



Figure 3. Alcoholic extraction of capsaicin.

## Detection of capsaicin by TLC

The extracted capsaicin was separated on silica gel 60 plates with a concentration zone (Merck) from two sources: commercial red hot pepper powder and Iraqi red hot pepper. It was then applied to the commercial and Iraqi red hot peppers that separated on TLC plates and created to study the capsaicin by exposure to UV light. The mobile phase used for separation is Toluene: Ethanol in different percentages as follows (7:3), (8:2), and (9:1) with close  $R_f$  as shown in Table (1). [13]

## Agar well diffusion bioassay of ethanol extract

The agar well diffusion method was used to evaluate the antibacterial activity of ethanol extract using pure cultures of every species of bacteria. For 22 hours at 37°C, the investigated microorganisms were grown in stock cultures in Muller Hinton Broth (MHB, Merck, Germany) medium. The McFarland turbid meter was used to calibrate the final cell concentrations to 1.5 10<sup>8</sup> CFU/ml. [14, 15] Using a cup borer (6 mm) into the agar, wells were made in the seeded agar plates after the media had had time to harden. Three wells were then created in each agar plate containing the tested bacteria, and 100µL of extract dilutions (500 mg/10 mL, 200 mg/10 mL, and 125 mg/10 mL) were added to the wells. The negative control in this case was ethanol. After a 24-hour incubation period at 37 °C, the diameter of the inhibition zone was used to determine the antibacterial activity of the plates. The size of the inhibition zone that forms throughout the well determines how well the antimicrobial action is evaluated.

## RESULTS AND DISCUSSION

The technique of TLC was used as the first evidence to confirm the presence of Capsaicin in red hot Iraqi pepper. Results obtained from the (TLC) of the ethanol extract confirmed the presence of Capsaicin in the plant extract in comparison with standard Capsaicin as the  $R_f$  value of the Capsaicin in the extract and the standard is very close in three different mobile phases as represented in Figure (4) and Table (1) below:

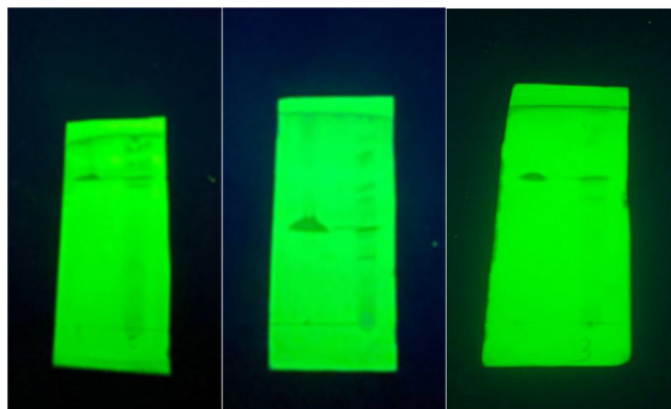


Figure 4. Extracted capsaicin compared to the standard capsaicin in the following mobile phases: (toluene: ethanol) (7:3), (8:2) and (9:1), respectively

Table 1.  $R_f$  values of ethanolic extract Capsaicin

Solvent system	$R_f$ value of standard capsaicin	$R_f$ value of ethanol extract capsaicin
Toluene: ethanol 7:3	0.75	0.74
Toluene: ethanol 8:2	0.65	0.64
Toluene: ethanol 9:1	0.47	0.46

Capsaicin extract from red hot Iraqi pepper was screened for its antimicrobial activity via agar well diffusion method and ethanol was used as a negative control against G- (*E.coli* and *K. spp.*) and G+ (*S. aureus* and *S. epidermidis*) and *Candida albicans* at different concentrations of (500 mg/ 10ml, 250 mg /10mL and 125 mg/10mL) of ethanol extract as shown in Table

(2), Figure (5) and Figure (6). The alcoholic extract of Capsaicin showed variable results at three different concentrations, as it had the highest inhibitory action against *Staphylococcus epidermidis* with the average diameter of the inhibition circle diameter 16.0 mm at the concentration of 500 mg/10 mL, while a moderate inhibitory action against *S. aureus* and *Candida albicans*.

**Table 2. Antimicrobial activity of capsaicin ethanolic extract with different microorganism species measured in millimeter by agar well diffusion method**

Bacterial species	Concentration in milligram/ millileter inhibition zone in millimeters		
	500 mg/mL	250 mg/mL	125 mg/mL
<i>Staphylococcus aureus</i>	13 S	12 S	11 S
<i>Staphylococcus epidermidis</i>	16 S	10 S	R
<i>Escherichia coli</i>	14 S	9 S	R
<i>Klebsiella sp.</i>	13 S	R	R
<i>Candida albicans</i>	13 S	10 S	11 S

Where R: resistance, while S: sensitive. [15]

Most sensitive comparisons with *E.coli* because of high resistance bacteria to the ethanol extract Figure 6.



**Figure 6. Inhibition efficacy of capsaicin ethanolic extract against gram-negative bacterial species *E.coli* measured in millimeter by agar well diffusion method**

In this study, the ethanolic extraction of the red hot Iraqi pepper showed a good to moderate inhibitory efficacy against the studied microbial species and other research around the world support this finding. [17, 16] The application of plant metabolites as antibacterial agents has been a focal point of microbiological and phytochemical research in recent decades. [18] Capsaicin has potent action against the bacteria *S. aureus*. [19] In particular, it has been demonstrated to impact staphylococcal cell viability, displaying bactericidal effects ranging from partial to complete, contingent upon the type under investigation and the degree of dilution. [20] It has been demonstrated that capsaicin somewhat inhibits the growth of *Escherichia coli* O157:H7 bacteria. [20] Numerous studies have examined *Candida* spp.'s susceptibility to capsaicin, with encouraging findings. Capsaicin has significant anti-*Candida albicans* inhibitory effects. [21]

## Conclusion

Capsaicin was extracted as an alcoholic extract from red hot Iraqi pepper and was confirmed by TLC. The antimicrobial activity of the ethanolic extract against different microorganisms showed a good to moderate inhibitory efficacy against the tested bacterial isolates and *candida albicans*. The highest inhibitory action of the plant's extract was against *Staphylococcus epidermidis*, while a high resistance appeared against *E.coli*.

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