

Effect of crude methanolic extract of Cuminum cyminum on growth of some types of pathogenic bacteria

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Abstract

Crude methanolic extract of cumin was examined for its antibacterial activity against some pathogenic bacteria namely ; Staphylococcus aureus , Salmonella typhi , Escherichia coli , and Pseudomonas spp . At varying concentration of 400,600,800 mg/ml using disk diffusion method as preliminary screening . The results showed an inhibitory effect of the extract on the activity of the tested microbes . This was reflected by the minimum inhibitory concentration of Staphylococcus aureus , Salmonella typhi , Escherichia coli and Pseudomonas sp. Which was (25,25,100,100,mg/ml respectively) . Also the minimum bactericidal concentration to the bacteria mentioned above was (50,50,200,200 mg/ml respectively) . In addition ,the present study show that cumin extract is equally effective against both gram-positive and gram-negative bacteria.

1.Introduction

Finding healing powers in plants is an ancient idea People on all continents have long applied poultices and imbibed infusions of hundreds , if not thousands , of indigenous plants , dating back to prehistory . There is evidence that Neanderthals living 60.000 years ago in present day Iraq used in ethnomedicine around the world . Historically , therapeutic results have been mixed , quite often cures or symptom relief resulted . Poisonings occurred at a high rate , also. However since the advent of antibiotics in the 1950 , the use of plant derivatives as antimicrobials has been virtually nonexistent [1] .

In this respect spices are used as culinary ingredient for taste and tang . They are also used in oriental medicine [2,3] .

The antibacterial activities of spices and essential oils have been known for a long time, and a number of researches on the antibacterial effect of spices, essential oils and their derivative have been reported [2,4,5,6,7,8,9,10,11,12,13,14] .

Among the most popular herbal medicine is cumin which is used world wide . It's botanical name is Cuminum cyminum , for the umbellifera family . It was especially sought after in the world , and was used as condiment , and medicine by the Babylonian and Egyptian people . The Egyptian not only used the spice to aromatize meats, fish and stews , but also to mummify their dead [2] .

There are two reasons to be interested in antimicrobial plant extract : **first** , world wide spending on finding new anti-infective agents is expected to increase 60% from the spending level in 1993 , **Second** : the use of plant extracts , as well as other alternative form of medical treatments , is enjoying great popularity in the late 1990 [1] .

This study aims to examine the effect of antimicrobial activity of methanolic extract from cumin on gram-positive and gram-negative bacteria .

2. Materials and methods .

2.1 Bacterial strains .

All bacterial species were obtained from health laboratory of the ministry of health .

2.2 Culture preparation .

The bacterial suspension was prepared by transfer one pure colony to (10 ml) bottle containing sterilized Nutrient broth , the bacterial was incubated at (37 °C) for (18 h) . Turbidity at 600nm of bacterial suspension was adjusted to given approximately (1.5×10⁸ cfu/ml) [15] by using spectrophotometer ' McFarland standard ' .

2.3 preparation of crude methanolic extract .

A fifty mg of cumin seeds was ground in an ammixer and extracted for (6h) . In a soxhlet extractor with (250 ml) methanol at (70%) . The crude extract was pooled , filtered and then concentrated by distributed the extract in a sterile petri dishes and then incubated in incubator at (37 °C) .

Stock solution of crude methanolic extract was prepared by diluting the dried extracts with (10%) dimethyl sulfoxide (DMSO) solution to obtain a final concentration of (800 mg/ml) .

2.4 Screening of extract using disk diffusion technique .

The disk diffusion test was performed using the standard procedure as described by [10] . The inoculum suspension of each bacterial strain was swabbed on the entire surface of Nutrient Agar. Sterile (6 mm) filter paper discs were aseptically placed on NA surface and crude methanolic extract was immediately added to discs in volumes (20 µl) .

A (20 μ) aliquot of (10%) DMSO was also added to a sterile paper disc as a negative control, whereas disc containing (30mg) amoxycilin was placed in the plate as apposite control. The plates was left at ambient temperature for (15 min) to allow excess prediffusion of extract prior to incubation at (37 $^{\circ}$ C) for (24 h). Diameters of inhibition zone were measured. Four replicates were produced for each bacteria.

2.5 Determination of the minimum inhibitory concentration and minimum bactericidal concentration using microbroth dilution test.

The dilution test was performed to determine minimum inhibitory concentration (MIC). using the standard procedure as described by [10]. (50) microleter of Muller Hinten Broth (MHB) were added in each test tube. The (50 μ) aliquot of stock solution of crude methanolic extract (800 mg/ml) was added and subsequently two fold serialy diluted with (MHB). The inoculum suspension (10 μ) of each bacterial strain was then added in each tube containing crude methanolic extract and (MHB). The final concentrations of the extract were 400,200,100,50,25,12.5,6.25 mg/ml.

The negative control was also performed using 10% (DMSO). Duplicate tubes were run for each concentration of spice extract. The tubes were incubated at (37 $^{\circ}$ C) for (24h), and the turbidity was measured at (600 nm) by using spectrophotometer. The lowest concentration that inhibited visible growth of the tested organism was recorded as the (MIC). The counting was done to all bacterial colonies, and the value of (MBC) was identified. Where; the low concentration of extract component inhibit the growth or given less than three colonies compared with our control.

3. Results and discussion :-

3.1 preliminary screening of spice extract :-

The result of disk diffusion test indicated that cumin showed different degrees of growth inhibition, depending on the bacterial strains (Table 1):-

Table 1 :- Antibacterial activity crude methanolic extract of cumin against bacteria using disk diffusion test.

Organisms	zone inhibition(mm) ^a				
	400	600	800	DMOS	Amox
<u>Pseudomonas sp.</u>	15.5	16	18	_b	20
<u>Salmonella typhi</u>	16.5	18.25	20.75	_	28.75
<u>E. coli</u>	12.5	13.25	15	_	22.5
<u>Staph. aureus</u>	16	17	18.5	_	19.6

a : data mean of four replicate.

b : No inhibition was observed.

The methanolic extract of cumin showed the broadest antibacterial activity by inhibiting growth of all bacterial strains tested. Both gram-positive and gram-negative bacteria were sensitive to the

Salmonella typhi, followed by (18.5, 18 mm diameter of zone of inhibition) at 800 mg/ml against Staphylococcus aureus and Pseudomonas sp. respectively. The least activity (15mm diameter of zone of inhibition) was demonstrated against E.coli at 800 mg/ml. No obvious difference in susceptibility was found between gram-positive and gram-negative bacteria. There was no inhibition of growth with the vehicle control (10% DMSO).

3.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Table (2) shows the results of determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results showed that the least (MIC) and (MBC) values of 25 mg/ml and 50 mg/ml respectively with Salmonella typhi. While the highest values of 100 mg/ml (MIC) and 200 mg/ml (MBC) against E.coli and Pseudomonas spp.

Table (2) :-

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of cumin against the test organisms.

Organisms	MIC mg/ml	MBC mg/ml
<u>Pseudomonas sp.</u>	100	200
<u>Salmonella typhi</u>	25	50
<u>E.coli</u>	100	200
<u>Staph. aureus</u>	25	50

Plant essential oils and extracts have been used for many thousands of year, in food preservation [2,9], alternative medicin [16]. In recent years, There have been arising interest in the discovery of new antimicrobial compounds, due to an alarming increase in the rate of infection with antibiotic resistance microorganisms [17]. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care [16].

Cumin was shown to have an inhibitory effect against Staph.aureus, Salmonella typhi, E.coli and Pseudomonas spp. In a study on antimicrobial activity of volatile oils of some spices [5,18,11] demonstrated that cumin had inhibitory effect against St.aureus, [18,10,11] proved an inhibitory effect against E.coli while [10,11] demonstrated an inhibitory effect against Salmonella typhimurium.

The antimicrobial activity has been attributed to the presence of some active constituents in the oils [5,1]. Phytochemical in cumin have been used as antioxidant [19], antibacterial agent and antifungal agent [1, 2,3,5,10, 20,21,22]. Cumin possesses numerous medicinal properties it is an aromatic herb and astringent that benefits the digestive apparatus, and acts as a stimulant of sexual oranges. It has been used in the treatment of mild digestive disorders, as a craminative, eupeptic and astringent, in broncopulmonary and as a cough remedy, as well as an analgesic.

cumin is useful in diarrhoea and dyspepsia treatments . It can be used topically in the form of a cataplasm in the elimination of stains and occasional pains. In a series of tests, Indian scientists have demonstrate that cumin increases glutathione transferase activity , which protects against certain types of cancer , further more cumin can decrease the chromosomal damage that could normally be caused by a powerful cancerous chemical agent , by (83%) , [2,10,23] .

The major constituents of cumin oil is : cumin aldehyde (20.72%) and monoterpene hydrocarbons (e.g *B*.pinene , γ , terpinene , *p*.cymene) [10] . DE etal [2] demonstrated that cumin aldehyde is effect against all the bacteria , fungi and yeast .

Plants have an almost limitless ability to synthesize aromatic substances . Most of which are phenols or their oxygen-substituted derivatives most are secondary metabolites .

In many cases , these substances serve as plant defense mechanisms against predation by microorganisms , insect , and herbivores [1] . According to the [11], the phenolic components of essential oils showed the strongest antimicrobial activity , followed by aldehydes , Ketones , and alcohols .

The mechanisms of responsible , for phenolic toxicity to microorganisms include enzyme inhibition by oxidized compounds , possibly through reaction with sulfhydryl groups or through more non specific interaction with proteins [1] .

Some researchers [1,5 ,10,24] demonstrated the antimicrobial activity of the most common terpene compounds , such as thymol , carvacrol , linalool , eugenol , X-pinene , and *B*.pinene in spices against several microbial strains . Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of motive force .

Cowan [1] reported that the antimicrobial action of spices is due to the impairment of a variety of enzyme systems involving in the production of energy or synthesis of struchural components in microbial cells. Sikkema etali [22] explain that an important characteristic of essential oils and their components is their hydrophobicity , which enable them to partition the lipide of the bacterial cell structures and rendering them more preamble .

Extensive leakage from bacterial cells or the exist of critical molecules and ions will lead to death [16] . Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria [5,12] . Yet the present study show that cumin extract is equally effective against both gram-positive and gram-negative bacteria . This result is further proved by prabuseenivasan etal yet . [16] in this experiment which evaluated the antibacterial activity of (21) plants essential oils against (6) bacterial spices . The variation in he present resultes and the pervious reports may be attributed to the different environmental growth conditions of plants and microbial spices [3] .

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تأثير المستخلص الكحولي المثلي لنبات الكمون *cuminum cyminum* في نمو بعض الانواع البكتيرية المرضية

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المستخلص

اجري البحث على المستخلص الكحولي المثلي لنبات الكمون *cuminum cyminum* لدراسة تأثيره في نمو الأنواع البكتيرية *Escherichia coli*، *Salmonella typhi*، *Staphylococcus aureus* و *Pseudomonas spp* وبطريقة الانتشار عن طريق الأقراص كمسح أولي وبتراكيز مختلفة (800,600,400) ملغم / مل . اظهرت النتائج ان للمستخلص الكحولي تأثيرا مثبطا تجاه نمو البكتريا المختبرة ، حيث كان التركيز المثبط الأدنى (MIC) للأنواع البكتيرية

Pseudomonas spp.، *Escherichia coli*، *Salmonella typhi*، *Staphylococcus aureus* كالآتي

(100,100,25,25) / مل على التوالي فيما كان التركيز القاتل الأدنى (MBC) للأنواع البكتيرية اعلاه .
(200,200,50,50) ملغم / مل (على التوالي . كما اظهرت الدراسة الحالية ان للمستخلص الكحولي نفس التأثير على البكتريا الموجبة والسالبة لصبغة كرام .