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Research Article

## Antimicrobial action of oregano and cyprus oils extract against *Klebsiella pneumoniae* bacteria isolated from different clinical samples

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### Abstract

Essential oils are concentrated natural extracts from plants containing bioactive components that have been demonstrated to have antioxidative and antibacterial properties. Methods: This study was focused on the antimicrobial activity of naturally *Cyperus rotundus* and *Origanum vulgare* oils extracted by following the hydro distillation process against fifteen *K. pneumoniae* clinically isolates. Well diffusion method was used for testing the antibacterial activity of oregano and cyprus oils extract against studied bacteria under four different concentrations (100%, 50%, 25%, 12.5%). Between these two essential oils, Oregano oil revealed a wide antibacterial spectrum against *K. pneumoniae* bacteria strains represented by measuring the zone of inhibition (ZIHs) area of growth. Oregano oil with 100% revealed the antimicrobial activity against *K. pneumoniae* which was isolated from burned samples with (67.0±2.64) milli metre inhibition zone diameter, under the 50 % oregano oil observed the antimicrobial activity against *K. pneumoniae* which isolated from stool with (30.16±3.12) mm. Other concentrations of oregano oil extract showed different scores of inhibition zone diameter, this differences statistically was ranging from highly significant to non-significant (P-value ≤, 0.05, ≥0.05). While Cyperus oil extract showed no antimicrobial activity against selected bacteria, Antibiotic susceptibility tests were applied to test the resistance status of all of the *K. pneumoniae* isolates and to compare the activity of oils with action of bacteria under the six types of antibiotics that belong to the Extended spectrum beta-lactamase (ESBLs) group. Such differences in susceptibility between the oil extracts and antibiotics are notable. Conclusions: Natural oil extract may provide an alternatives and supplements to conventional antimicrobial additives in foods or applying locally in clinical cases.

**Keywords** *Cyperus*, ESBLs, Hydro distillation, *K. pneumoniae*, *Oregano*

### Introduction

The development of novel antimicrobials is one of the measures being taken to combat the growth of antibiotic resistance. Essential oils from plants are being investigated as a potential replacement for currently used antimicrobials, and many plants essential oils have been found to exhibit significant antimicrobial activity. Their ability to manufacture aromatic compounds, the bulk of which are phenols or

oxygen-substituted derivatives, is credited with this action. Plants have been utilized for antibacterial, antiseptic, and other therapeutic purposes from prehistoric times, and have been widely used by all cultures for millennia ((Fakim, 2006). Interestingly, the World Health Organization (WHO) estimates that about 80% of the world's population, especially in developing nations, still uses medicinal plants and extracts for primary health care (Prabuseenivasan et al., 2006, Valiakos et al., 2015). The contemporary pharmaceuticals, cosmetics, and food businesses are built on the understanding of medicinal

plant qualities that can be employed for everything from treating infectious, systemic, and inflammatory disorders to food preservation and production of perfume (Hammer et al., 1999). Since antiquity, oregano has been used as a condiment, and its essential oil is known for its antibacterial and antispasmodic properties (Nostro et al., 2004). According to research on the antioxidant qualities of oregano, due to its high level of thymol and carvacrol contents, it has the strongest antioxidant capacity and has extraordinary effects in preventing fat oxidation (McKay et al., 2006). The essential oils of oregano have been found to possess highly bioactive chemicals with insecticidal and acaricidal properties (Cetin et al., 2009). Furthermore, it has potent antibacterial properties against foodborne and food-spoilage microorganisms (Mith et al., 2014; Nabavi et al., 2015). The medicinal qualities of the *Cyperus rotundus* rhizomes essential oil investigated in this study are frequently used in traditional medicine to heal stomach problems, wounds, boils, and blisters. This plant has been shown to have anti-candida, anti-inflammatory, antidiabetic, antidiarrheal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic and apoptotic, anti-pyretic, and analgesic pharmacological and biological effects (Jabier et al., 2008; Sivapalan, 2013). Therefore, this study was designed to assess and investigate the antibacterial action of oregano and cyperus oil against *K. pneumoniae* bacteria in vitro assay using the disk diffusion method and test the antibiotic resistance pattern of these isolates under the action of Extended spectrum beta-lactamases (ESBLs) antibiotic groups of clinically isolated *K. pneumoniae*.

## Materials and Method

### Collection of samples

Fifteen types of *K. pneumoniae* bacteria strains were collected from different clinical samples and diagnosed using VITEK techniques.

### Collection of the plants material

For extracting the oils from *Cyperus rotundus* and *Origanum vulgare* plants, the fresh rhizomes of dried leaves of both plants were bought from the local market in Baghdad city and were subjected to an extraction process.

### Extraction of essential oils

The Hydro distillation process was used to extract essential oils from both plant species (oregano and cyperus) using a Clevenger-type device (Yagi et al., 2016). Fresh *C. rotundus* rhizomes were cleaned of mud, and dirt, and were sliced into small pieces in a coffee blender; whereas, dried *Oregano* leaves were crushed into powder. One hundred grams (100 gms) of each plant separately were immersed in (1000 ml) distilled water in a conical flask and extracted by using the hydro distillation principle for (3-7) hours (Al-Oqail et al., 2016). After extraction, the oils were dried over anhydrous sodium sulphate. The oils were kept in amber-colored bottles

at (4 °C) to avoid the detrimental effects of light until usage (Kulaksiz et al., 2018).



Fig. 1: A; Oregano leaves B; Cyprus leaves

### Assessment of antibacterial action of essential oil against *K. pneumoniae* isolates

The antimicrobial activity of *Oregano* and *Cyperus* against isolated *K. pneumoniae* (n=15) bacteria was applied following the well-diffusing agar method.

### Agar-well diffusing test

The *K. pneumoniae* bacteria were re-streaking over a Muller Hinton agar plate. From the cultured bacteria, some pure colony was transferred using a cotton swab to a tube containing 5 ml of sterile normal saline and the turbidity of the bacterial suspensions was adjusted using a densitometer (Denis CHEK plus, Germany) using 0.5 MacFarland value as a standard reference which is equal to  $(1.5 \times 10^8)$  C.F.U/ml bacterial load (Monica, 2006). A sterile cotton swab was dipped in the tube containing bacterial suspensions and spread like carpet method over Muller Hinton agar plates, with the aid of a sterile cork borer, a well of (8) mm wide was made in the plates. One hundred microliters from the oils diluted in (DMSO) were transferred to each well. Four diluents of each oil were used: 100%, 50%, 25% and 12.5% concentrations. All the plate was left at room temperature for half an hour to allow diffusing of extract oils. After this process, all the plates were incubated at 37°C for (18-24) hours. At the end of the incubation period, the inhibition zone diameters of each concentration against each of the *K. pneumoniae* isolates were measured using a ruler. The experimental test was performed in triplicate.

**Antibiotics sensitivity test of *K. pneumoniae* isolates against ESBLs group**

After adjusting the turbidity of the bacterial suspension of *K. pneumoniae* isolates using a densitometer (Denis CHEK plus, Germany) using 0.5 MacFarland value as a standard reference which is equal to (1.5\*10<sup>8</sup> C.F.U/ml) bacterial load, a sterile cotton swab was dipped in the tube containing bacterial suspensions and spread like carpet method over Muller Hinton agar plates. A group of six types of antibiotics

disks belonging to (ESBLs) group was applied to each plate with sterile forceps (Table 3). All of the plates were incubated at (37±2 °C) for 24 hrs. under aerobic conditions. The sensitivity status of each antibiotic against *K. pneumoniae* isolates was interpreted according to the Clinical Laboratories Standards Institute (CLSI) after the incubation of all the plates at (37±2 °C) for 24 hrs. A ruler was used as a tool for measuring the inhibition zone of antibiotics.

**Results**

**Table 1. Isolation of bacterial species from clinical samples**

No	Name of bacteria	Sample	N	%
1	<i>K. pneumoniae</i>	Urine sample	8	53.3
2	<i>K. pneumoniae</i>	Stool sample	2	13.3
3	<i>K. pneumoniae</i>	Blood sample	2	13.3
4	<i>K. pneumoniae</i>	Sputum sample	1	6.6
5	<i>K. pneumoniae</i>	Ascites fluid	1	6.6
6	<i>K. pneumoniae</i>	Burn sample	1	6.6
Total			15	(100%)
*P-value			0.02 (S)	

\*; Fisher exact test; S; significant

Table (1) demonstrated that 8 (53.3%) of *K. pneumoniae* bacteria were isolated from urine, while 2 (13.3%) were isolated from stool and blood samples respectively; statistically, this difference was significant (P-value= 0.02).

**Table 2. Frequency and percentage of ESBLs antibiotic resistance among *K. pneumoniae* isolates**

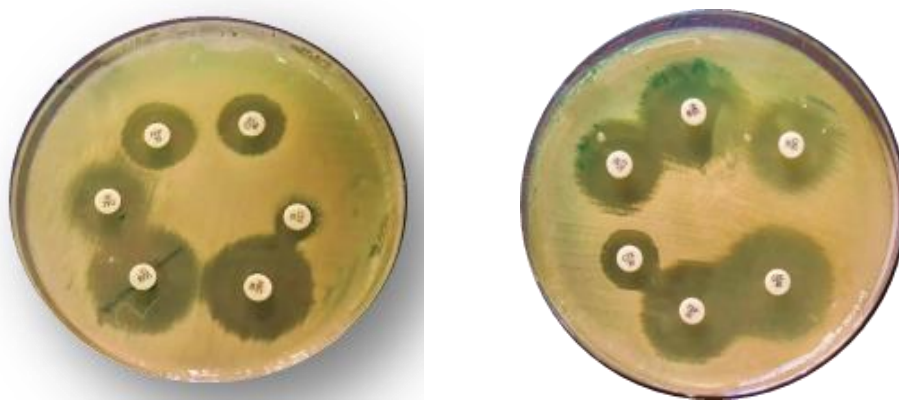
Types of ESBLs pattern	N	100%
ESBLs positive <i>K. pneumoniae</i>	14	90
ESBLs negative <i>K. pneumoniae</i>	1	10

**Table 3. Results of antibiotic sensitivity test of clinically isolates *K. pneumoniae* (N=15)**

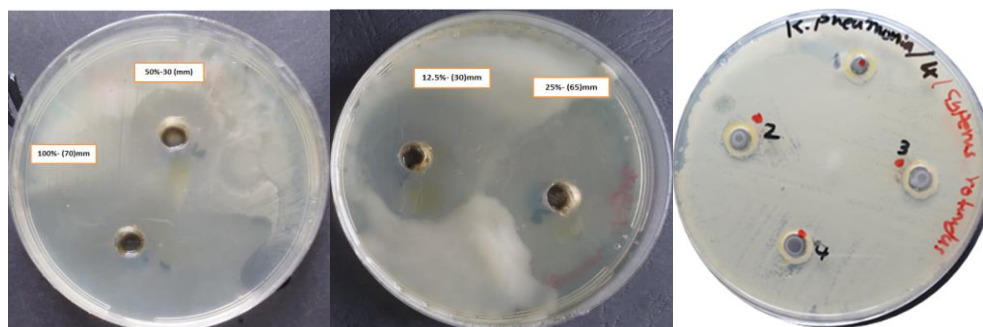
No.	Name of bacteria	Sample	CTX	CTC	FEP	FEC	CAZ	CZ C	ESBLs Result
1	<i>K. pneumoniae</i>	Urine	10	7	R	R	R	5	+VE
2	<i>K. pneumoniae</i>	Urine	R	1	R	25	25	30	+VE
3	<i>K. pneumoniae</i>	Urine	R	15	2	2	15	20	+VE
4	<i>K. pneumoniae</i>	Urine	R	10	10	16	10	19	+VE
5	<i>K. pneumoniae</i>	Urine	R	R	R	14	R	15	+VE
6	<i>K. pneumoniae</i>	Urine	R	R	R	R	R	R	-VE
7	<i>K. pneumoniae</i>	Urine	10	20	25	25	15	20	+VE
8	<i>K. pneumoniae</i>	Urine	15	14	15	20	15	20	+VE
9	<i>K. pneumoniae</i>	Stool	30	25	26	30	25	25	+VE
10	<i>K. pneumoniae</i>	Stool	13	20	23	25	20	25	+VE
11	<i>K. pneumoniae</i>	Blood	13	17	20	22	15	25	+VE
12	<i>K. pneumoniae</i>	Blood	20	20	20	25	20	25	+VE
13	<i>K. pneumoniae</i>	Sputum	8	15	15	2	2	25	+VE
14	<i>K. pneumoniae</i>	Ascites fluid	R	R	15	20	R	R	+VE
15	<i>K. pneumoniae</i>	Burn	7	15	20	25	15	25	+VE

**Table 4. Means of the zone of inhibition of *Oregano* oil with different concentrations (100%, 50%, 25%, 12.5%) against *K. pneumoniae* isolated from different clinical samples (N=15)**

Conc of Oregano	Samples	N	Mean±SD	Maximum	Minimum	*P-value
<b>100%</b>	Urine	8	22.50±9.50	35.00	0.00	<b>&lt;0.001</b>
	Blood	2	11.66±12.81	25.00	0.00	
	Stool	2	23.83±7.02	35.00	17.00	
	Burn	1	67.0±2.64	70.00	65.00	
	Sputum	1	29.0±1.0	30.00	28.00	
	Ascites fluid	1	23.3±1.52	25.00	22.00	
<b>50%</b>	Urine	8	19.66±8.53	30.00	0.00	<b>0.27 (N.S)</b>
	Blood	2	14.5±15.89	30.00	0.00	
	Stool	2	30.16±3.12	35.00	26.00	
	Burn	1	26.66±4.16	30.00	22.00	
	Sputum	1	29.66±0.57	30.00	29.00	
	Ascites fluid	1	23.0±2.64	25.00	20.00	
<b>25%</b>	Urine	8	20.41±8.78	30.00	0.00	<b>&lt;0.001</b>
	Blood	2	11.83±12.99	25.00	0.00	
	Stool	2	25.83±3.06	30.00	22.00	
	Burn	1	27.0±3.60	30.00	23.00	
	Sputum	1	66.0±4.0	70.00	62.00	
	Ascites fluid	1	22.66±2.51	25.00	20.00	
<b>12.5%</b>	Urine	8	32.83±36.05	70	0.00	<b>0.9 (N.S)</b>
	Blood	2	24.33±0.81	25.00	23.00	
	Stool	2	28.66±1.52	30.00	27.00	
	Burn	1	23.0±2.0	25.00	21.00	
	Sputum	1	23.33±1.52	25.00	22.00	
	Ascites fluid	1	26.31±18.18	27	26	



**Fig 2. Results of antibiotic sensitivity test of *K. pneumoniae* isolates against ESBLs group**



**Fig. 3.** Results of antimicrobial action of oregano and cyprus oils against *K. pneumoniae* isolates.

Table 2, Table 3 and figure 1 documented that 14 (90.0%) out of 15 *K. pneumoniae* bacteria were resistant to (ESBLs) antibiotics which were indicated by an increased score of inhibition zone diameter  $\geq 5$  mm higher with clavulinic acid than without, while only 1 (10%) isolates phenotypically not founded resistance to (ESBLs) antibiotics. Also, the results of this study documented that the most resistant isolates with (ESBLs) antibiotics were those isolated from urine sample 5 out of 7 isolates.

Table (4) and figure (2) revealed that 15 clinically isolated *K. pneumoniae* were differently sensitive when treated with two types of natural oil extracts. This table showed that the oregano oil with absolute concentration (100%) has the highest antimicrobial action against *K. pneumoniae* which isolated from burn sample 1(6.6%) which scored inhibition zone ( $67.0 \pm 2.64$  mm) maximum zone of inhibition read 70 mm and minimum inhibition zone read 65 mm, under the same concentration (100%) the *K. pneumoniae* which picked up from blood 2 (13.3) scored ( $11.61 \pm 12.81$ mm) with a maximum zone of inhibition read 25 mm and minimum zone of inhibition read zero. By decreasing the concentration of natural oregano oil from 50 % to 12.5%, it still gives antimicrobial activity against most *K. pneumoniae* isolates with different ranges of inhibition zones; statistically, this difference under different concentrations of oregano oils was with different significant ranging from highly significant (P-value= $<0.001$ ) to non-significant (P-value= 0.9). All of the 15 *K. pneumoniae* isolates were resistant and showed no inhibition zone under a different concentration of Cyperus oil (100%-12.5%).

## Discussion

Many years ago, plants were used for many aims, ranging from treating infectious diseases to food preservation. Currently, due to the increase in the resistance of microorganisms to many types of antimicrobials used in combination with the appearance of new diseases needs the urgent development of new, and more active drugs. Plants are a unique and renewable source for the development of new antibacterial, antifungal, and antiparasitic chemicals due to

the biological and structural diversity of their constituents (Sakkas & Papadopoulou, 2017). A total of 15 *K. pneumoniae* clinically isolates was subjected to antimicrobial sensitivity under the action of two different plants extracted with the hydro distillation process. The result of this revealed that the antimicrobial activity of oregano oils was observed against all the isolated *K.pneumoniae* from different clinical sources with different scores of inhibition zones, these findings agreed with (Sakkas & Papadopoulou, 2017, Hadad, & Samanje 2022) who published the oregano oil, thyme oil and basil oils showed a different score of effects ranging from sufficient to moderate effects against multi-drug resistance clinical bacteria. Both thyme and oregano essential oils are proven to have significant antibacterial activity. This activity is attributed to some phenolic components that exist in them (Yamazaki et al., 2004). In one study, oregano was the most active oil out of 52 essential oils against a panel of gram negative and gram positive bacteria, these include (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacterbaumannii*, *Aeromonas sobria*, *Enterococcus faecalis*, *Salmonella Typhimurium*, *Serratia marcescens* *S. aureus*), which inhibiting all these bacteria under the concentration  $<2\%$  (v/v) (Hammer et al., 1999). Regarding the antimicrobial activity of Cyperus rotundus, there is no antimicrobial activity against all clinically isolated *K. pneumoniae* (n=15), the results of the current study agreed with study (Dadooka et al., 2019), which documented that cyperus rotundus possessed antimicrobial activity against *Klebsiella pneumoniae* isolated from urinary tract infection under the concentration (25 mg/ml). Because essential oils are high in phenolics, they have the potential to alter the permeability and function of cell membrane proteins by permeating the bacterial cell wall's phospholipids layer, attaching to proteins, and inhibiting their normal functions. Since essential oils are lipophilic materials, they can produce an effect on unsaturated fatty acids in terms of percentage and structure. However, due to the diversity of molecules present in plant extracts, their antimicrobial activity cannot be attributed to a single mechanism, but rather to several different mechanisms affecting the functions of the cell membrane, cytoplasm, enzymes, proteins, fatty acids, ions, and metabolites at various sites of the bacterial cell outer and

inner components (Sakkas & Papadopoulou, 2017) .In conclusion, *Origanum vulgare* extract possesses an antimicrobial effect and thus inhibits the growth of *K.pneumoniae* bacteria and using great potential in treating this type of bacteria when clinically causing infection.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication

### Ethical Considerations

The study was approved by the institutional ethical committee

### Funding support

The author declares that they have no funding support for this study

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