MENOUFIA JOURNAL OF AGRICULTURAL BIOTECHNOLOGY

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PROMOTING ACTION OF ANTIBIOTICS USING THYME OIL FORMS AGAINST RESISTANT BACTERIA CAUSING URINARY TRACT INFECTION

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Received: Mar. 17 , 2024 Accepted: Mar. 30 , 2024

ABSTRACT: In the last decade, the increased rate of resistance to antibiotics has been critical barrier to successful treatment of various bacterial diseases. Given the increasing antibiotic resistance in urinary tract infection (UTI), alternative strategies need to be examined. In the current work, four main bacteria were isolated from urinary tract infected patients. The isolates were biochemically characterized as *Escherichia coli, Klebsiella pneumonia, Staphylococcus saprophyticus and Enterobacter faecalis*. These UTI bacterial isolates differed in their resistance to the 12 used antibiotics with percentages between 56-100% and therefore cannot be used for empiric therapy in many geographic regions. Thyme oil showed some active compounds such as thymol, P-cymene, Carvacrol, Cineol and Linalool with different percentages when examined by GC-ISQ mass spectrometry. Addition of thyme oil and thymol crystals in the form of emulsion and nano-emulsion (2.5%) to the used antibiotics against resistant UTI isolates showed synergistic action as the *E.coli* isolate that were resistant to β-lactams, aminoglycosides and glycolipids antibiotics get affected and become susceptible to used antibiotics. Moreover, the concentrations of thyme oil or thymol crystal used to reach the MIC and MBC were reduced by 50% when nano-forms of either compounds were applied. While transmission electron microscope (TEM) image of *E.coli* isolate treated with nanoform of thymol crystals (2.5%) revealed disruption and phenotypic changes of cell wall compared with untreated control, and therefore improve bacterial penetration and increasing the activity of antibiotics and prove the synergistic action.

Key words: Thyme oil; Thymol crystal; Nano-emulsion; UTI pathogens.

INTRODUCTION

The misuse and overuse of antibiotics in health care, human, and veterinary treatment and poultry farming has helped and led to the emergence of antibiotic resistance, which spread and developed rapidly, leading to a decrease in its effectiveness in diseases treatments and reduces the therapeutic options available to the medical community against these bacteria. This leads to an increase in infection with antibiotic-resistant pathogens, which increases morbidity and mortality (Centers for Control of Diseases and their prevention, 2019). In this regard, multidrug-resistant pathogens are becoming more common in community-acquired infections Koulenti *et al*., (2020) and one of the most important reasons for the emergence and development of antibiotic resistance is their extensive use of traditional treatments especially in veterinary medicine, animal feed, fish farming, and agricultural use Smith *et al*., (2009). Moreover, contamination of soils, sediments and water bodies has also accelerated the development and spread of antibiotic-resistant strains in the environment Gothwal and Shashidhar, (2015) and Bin Saeed *et al*., (2016). The waste of pharmaceutical industries, hospitals, and livestock producers who produce large, uncontrolled quantities of antimicrobials induces multidrug resistance in the environment with potential of resistance spreading to humans and animals Oyekale, (2017). Antimicrobial resistance (AMR) has become a major health concern well recognized by major bodies

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worldwide, including the World Health Organization (WHO) and the Centers for Disease Control and Prevention. The currently estimated annual global death rate attributable to AMR is 700,000, and this number is expected to escalate rapidly and reach the alarmingly high figure of 10 million deaths per year in 2050 according to (WHO) Talaat *et al*., (2022). Bacteria have four major characteristics and mechanisms that enable them to resist the action of antibiotics, and they can also develop and update them. These mechanisms include reduction of drug absorption; drug target modification; disable medication active drug flow; and the intrinsic resistance from drug inactivation Reygaert, (2018).

On other side, urinary tract infection (UTI) is one of the most important and common disease, as it occurs as a result of infection with bacteria and its multiplication in the urinary tract system. the most common cause of infection is *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus facials*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and other genera (Haghi, 2004). However *E.coli* bacteria is one of the most members of the enteric family that causes about 90% of urinary tract infections Jawetz *et al.*, (2016).

In light of the low effectiveness of current antibiotics and the development of mechanisms of bacterial resistance to antibiotics and the lack of discovery of new antibiotics, it was necessary to search different strategies to solve this problem and increase the effectiveness of existing antibiotics. This is becoming a concern of the researcher and attracted them to develop a drug fortified with antimicrobial compounds extracted from natural vegetable oils and other natural resources Mbaveng *et al.*, (2015) and Anand *et al.*, (2019). The synergistic combination of antibiotics and phytochemicals represents a promising strategy with many clinical and developmental benefits. Where some plant compounds have direct activity against antibiotic-resistant bacteria, others can sensitize antibiotic-resistant bacteria in different ways, such as facilitating entry into cells by destabilizing the cytoplasmic membrane (Lee *et al*., 2016; Bhattacharya *et al*., 2018); inhibition of efflux pumps (Kakarla *et al*., 2017) or biofilm dispersal (Skariyachan *et al*., 2018) among other mechanisms of action.

Thyme essential oil is a product of the steam distillation of fresh flowering aerial parts of thyme herb (*Thymus vulgaris* L), and their main volatile components; thymol (2-isopropyl-5-methyl phenol) and carvacrol (2-methyl-5-propanyl phenol) have found wide application for therapeutic objectives. The synergistic effect of essential oil and antibiotic combinations exceeds the sum of the individual effects of each. This synergistic action was studied to reveal the in vitro antimicrobial efficacy of thyme oil forms including nanoparticles against laboratory model of multidrug resistance *E.coli* Fahim and Hussein (2017).

In this study, thyme oil, its aqueous extract and nano aqueous extract as well as thymol crystals were selected and tested to support the traditional antibiotics, as thyme oil rich in phenolic thymol which has primary antimicrobial activity. The potential role of oil nano-emulsion against antibiotic-resistant pathogens, as well as the reversible ability of antibiotics-thymol oil combinations and its main active ingredient on various urinary tract infection (UTI) treatments were examined.

This study also was conducted with the aim of strengthening the action of antibiotics-formula of some natural resources of vegetable and aromatic essential oils (thyme oil) as natural resources to benefit from the synergistic action of them against antibiotic-resistant bacteria, as well as to change the usual resistance mechanisms of bacteria against some traditional antibiotics.

MATERIALS AND METHODS.

1. Isolation of Uriny Tract Infection UTI Bacteria

- Sampling: Urine samples (642) were collected From Emergency Teaching Hospital at El-Mansoura University under sterile condition from UTI patients who suffered from urinary

tract injuries and were admitted to the under specialized medical supervision during the period for one year. Urine samples were transferred to hospital microbiology unit and divided into two parts; the first part was handled immediately by centrifugation at 300 rpm for 3 min in a sterile container. Samples pellet and few drops of supernatants were mixed and microscopically examined (40X) by placing a drop of urine with a sterile pipette on a slide for calculating the percentage of pus cells according to Clinical and Laboratory Standards Institute CLSI (2017), as pus cells were taken as preliminary indication to determine inflammation rates. The second part of urine samples were used for isolation of pathogenic bacteria by streaking urine samples on nutrient agar (NA) (Oxoid, UK). The plates incubated at 37°C for 72 h Benito *et al.,* (2004).

- References strains: Two genetically modified strains; *E.coli* EMG01 and *E.coli* EMG02 were provided by the National Research Center (Molecular Genetics Laboratory, Biotechnology Research Institute, NRC, Egypt). The strain *E.coli* EMG01 is resistant to Ampicillin and Neomycin, and *E.coli* EMG02 is resistant to Ampicillin and Chloramphenicol Fahim and Hussein, (2017).

2. Characterization of UTI isolated bacteria

Pathogenic bacterial isolates were identified based on Gram staining and conventional biochemical reactions (urease, coagulase, catalase, oxidase, hemolytic action in blood agar and IMVC's tests based on the guideline recommendations of the Clinical and Laboratory Standards Institute CLSI guidelines, (2017).

3. The preparation of thyme derivatives

3.1. Thyme oil extraction and purification

Leaves of thyme (*Thymus vulgaris*) plants, Labiatea family were obtained from the Department of Horticulture at the Faculty of Agriculture, Menoufia University. Extraction was carried out using steam distillation by rotary evaporator at low temperature and under pressure. While thymol crystal (Active substance in thyme oil) obtained from Al-Gomhoria company with purity of 99.9% according to the procedure described by Guenther, (1960).

3.2. Thyme oil GC-Mass Spectrophotometry

The purified thyme essential oil was conducted using a Trace GC-ISQ mass spectrometric protocol (Thermo Fisher Scientific, Autosampler AS1300, USA) with a direct capillary TG-5MS column, the main components were detected by comparing their mass spectral and their retention times with those reported and stored on the MS library databases, respectively Sugumar *et al.,* (2014).

3.3. Preparation of specific emulsions of thyme oil and thymol crystal.

The emulsification process of thyme oil and thymol crystal were synthesized using highly purified crude oil or crystal substance. The non-ionic Polyoxyethylene-20-Onooleate scientifically named with ($Tween_{80}$) used as water emulsion surfactant obtained from (United chemicals and medicinal company, Cairo, Egypt). The emulsions were prepared under some physical modifications in the protocol by using 1 part of oil or crystal to 3 parts of $tween_{80}$ as emulsion agent according to the modified procedure devolved by Ghosh *et al*., (2013).

The nano-emulsion form preparation, the natural additives substances were slowly adding to distilled water up to 100 ml with gentle stirring using a magnetic stirrer for 45 min under cooling process. The nano-emulsion was subjected to a homogenizer (Wise Tis® homogenizer HG-15A; Daihan Scientific, Co., Ltd., Seoul, Korea),10.000 rpm for 30 minutes Droplet size and multiple scattering index (PDI) were measured using the dynamic light scattering (DLS) technique using a particle size analyzer (Malvern-UK, 4700) according to (Anjali *et al*., 2010 and El-Ekiaby, 2019).

4. Antimicrobial tests and promoting action

4.1. Antibiotic susceptibility testing

Primarily antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method according to the protocol described by the clinical laboratory standards institute (CLSI) guidelines using following standard antibiotics discs (Bio-Rad) as follow: Ampicillin (10 µg), Amoxicillin (10 µg), Cefoxitin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Erythromycin (10 µg), Gentamycin (10 µg), Imipenem (10 µg), Streptomycin (10 µg), Tetracycline (30 µg), Neomycin (10 µg), and Vancomycin (30 µg) for each antibiotic susceptibility disk. The UTI bacterial isolates were inoculated onto a Muller-Hinton agar (Oxoid), the antibiotic discs were placed on the surface of the agar plates. The plates were incubated for 24 h at 37°C until formed of bacterial film. After incubation, the diameter of the inhibition zones in millimeters was measured to assess resistance or susceptibility according to the interpretation criteria for *E.coli* (ATCC No. 25922) established by the (CLSI) guidelines. The multidrug resistance (MDR) is defined as resistance to more antibiotics belonging to different antibiotic classes CLSI guidelines, (2017).

4.2. Antibacterial activity of thyme oil and thymol crystal forms

Antibacterial activity test was determined by using Mueller-Hinton agar plates and carried out according to the protocol described by (CLSI) guidelines as follows: The overnight UTI bacterial cultures grown on Mueller-Hinton broth (Oxoid) were adjusted to the density of 0.5 McFarland turbidity standard unit. The inoculation of the tested bacteria was streaked on to Mueller-Hinton agar (Oxoid) plates using a sterile swab. Sterile filter discs (diameter 6 mm) (Whatman Paper No (1), England) were impregnated with (150, 200 and 250 µl/disc) of purified filtrated produced forms of (thyme oil and thymol crystal solution, respectively). The disks filled with serial concentration of 1.5, 2, and 2.5% of each component were placed on the appropriate agar medium, Ethanol 70% was used as positive control. After incubation at 37°C for 24 h, the diameters of the inhibitions zones in millimeters were measured. The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity, each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded with its standard divisions Vandepitte *et al.,* (2003). On other side, the Mueller-Hinton broth tubes were inoculated with 100 colony forming unit per mL (CFU/mL) of each UTI *E.coli* isolated strain and incubated at 37°C for 24 h. the minimal inhibitory concentrations (MICs) mg/mL were defined as the lowest concentrations of oil or crystal that completely inhibited the growth in the culture broth. While the growth control consisting of sterile media without growth (negative control), at the same dilutions was employed in the experiments to detect the minimum bactericidal concentration (MBCs) which were carried out to check whether the isolated UTI bacterial strains which were completely killed their growth when transfer to Mueller-Hinton agar plates. The obtained MBCs serial dilutions was then sub-cultured and incubated at 37°C for 24 h, each plate was observed for any colony growth on Mueller-Hinton agar according to the method described by Smith and Brown, (2017).

4.3. Promoting action of antibiotics using thyme oil and thymol crystal forms

Different concentrations of thyme oil and thymol crystal forms (10%) was conducted to promote the various antibiotics used in this study against the isolated UTI pathogenic bacteria. The sterile filtrated thyme oil and thymol crystal in the forms of emulsion and Nano-emulsion, were add to Mueller-Hinton media. The agar medium was poured into sterile petri dishes and were allowed to cool and solidify, which allowed to oil or crystal additives to homogenize within the culture medium, further progressive dilutions were used to obtain the final concentrations of (1.5, 2.0, and 2.5 %) of each oil and crystals emulsions and Nano emulsions forms. 1.5 .2.0 and 2.5 ml of these prepared solutions mixed with 10 ml of Miller Hinton media to obtain 1.5, 2.0 and 2.5

% concentrations, resp. Then the bacterial were spreading by sterile swab in the top of solidified agar by using one ml of treated overnight UTI bacterial cultures grown on Mueller-Hinton broth and adjusted to the density of 0.5 McFarland turbidity standard unit. The streaked tested bacterial plates were used as base of the antibiotic susceptibility testing which was re-performed using Kirby-Bauer disk diffusion method according to the protocol described by (CLSI) guidelines by using the previous each examined standard antibiotics discs. The plates were incubated for 24 h at 37°C. After incubation, the inhibition zones diameters in millimeters were measured to assess the promoting action of thyme oil or thymol crystal forms with studied antibiotics according CLSI guidelines, (2017).

5. Transmission Electron Microscopy (TEM) to treatment bacteria

The 24 h treated bacterial cells (CFU/ml) centrifuged at 10.000 rpm on 15 min, then pellets were collected en tube 20 ml and washed twice by sterile distilled water and kept into modified Karnovsky buffer solution (1 mM $CaCl_2$ 0.1%, 0.25 mg/ml glutaraldehyde and 0.25 mg/ml paraformaldehyde in 50 mM sodium cacodylate buffer pH 7). The bacterial cells were preserved in Eppendorf for no more than one week at 5°C, The observed films were transferred into a 0.1 mg/ml osmium tetroxide solution for one hour at 25°C and subsequently dehydrated by decimal graded acetone solution for 10 min. Moreover, Images of the treated by scanning isolated UTI *E.coli* by 2.5% thymol nano-emulsion were generated randomly, the bacterial film images were monitored using Corel Draw 12 magnifications, the gold sputter-coated in an SCD 50 sputter (Balzers) and observed with an EVO 40 XVP (TEM) (Electron Microscopy Unit, Mansoura University) as followed protocol Lanna-Filho *et al.,* (2017).

Statistical analysis

Each experiment was carried out in triplicate and the mean values were recorded with its standard divisions. The differences between values calculate by one-way analysis of variance (ANOVA). Probability value for the statistical test was 0.5 % was used to compare the differences of the inhibition zones, final data were subjected to statistical analysis using SPSS software (IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp).

RESULTS

1. Investigation of UTI urine samples.

Urine samples (642 cases) collected from patients suffered from urinary tract infections (UTI) and admitted to the hospital were subjected to turbidity observation, the results revealed that 458 out of 642 samples were turbid and showed bacterial growth on NA media which considered a pathogenic bacterium, while 184 samples showed no growth and light turbidity and considered free from urinary tract bacterial pathogens and may have another symptom. The cases differed among themselves in terms of the severity of the symptoms, clinical examination, and turbidity as well as the percentage of pus in the urine which examined by microscope (Table 1).

Table (1): Numbers and characteristics of UTI with pathogenic bacteria.

Number of Cases	Turbidity (NTU)	Pus cells numbers	Symptoms	Gram stain	12 antibiotics used	
					Resistance	Sensitive
170	2.43	50 cells >	+	G^{-ve}	2	10
19	2.39	50 cells >	+	G^{+ve}	3	9
160	4.52	100 cells>	++	G^{-ve}	3	9
11	4.41	100 cells>	++	G^{+ve}	3	9
91	7.15	500 cells>	+++	G^{-ve}	5	7
7	7.03	500 cells>	+++	G^{+ve}	6	6

Turbidity measurements was done by Nephelometric Turbidity Units (NTU)
(+) Minor, (++) Moderate and (+++) Severe clinical symptoms and pus cells per field.

2. Biochemical characteristics of pathogenic bacteria.

The results of biochemical characterization of pathogenic bacteria isolated from the 458 patient samples with urinary tract infections showed that 373 cases were infected with *Escherichia coli*, 47 cases with *Klebsiella pneumonia*, 10 cases with *Staphylococcus saprophyticus*. 3 cases with *Enterobacter faecalis*, and 25 cases with other causes. Biochemical identification of *S. saprophyticus* species is made on basis of the gram-positive reaction, Positive of hemolysin, coagulase, urease and catalases. The biochemical reaction that differentiates between *K. pneumoniae* species and *E.coli* species is urease test which is positive for the former while negative for *E.coli*. Moreover, *E.coli* was positive for indole production and methyl red, but negative for citrate and Voges - Proskauer tests Table (2).

Table (2): Biochemical characteristics of isolated (UTI) pathogenic causes.

No. cases	Microbial causses	Gram stain	Biochemical tests					IMVC's test				Percent %
			Hemolytic	Coagulase	Urease	Oxidase	Catalase	Vp	MR	Citrate	Indole	
373	*E.coli*	-	+	-	-	-	+	-	+	-	+	81.4
47	*K. pneumonia*	-	+	-	-	-	+	+	-	+	-	10.26
10	*S. saprophyticus*	+	+	+	+	+	+	-	-	-	-	0.02
3	*E. faecalis*	-	+	-	-	-	+	-	+	-	+	0.006
25	Other causes	V*	V*	-	V*	+	+	-	-	-	-	0.05
Total cases		458 UTI Cases						* V: Variable				

*Variable, some are positive, some are negative, and some genera are moulids.

3. Antibiotic resistance susceptibility testing.

Resistance to antibiotics was measured using antibiotics resistance CLSI scale, which assigned three levels of resistance (resistant, intermediate, and susceptible) for each antibiotic according to inhibition zone diameters caused by a specific antibiotic against standard *E. coli.* The antibiotics sensitivity tests against isolated pathogenic strains were conducted to determine the susceptibility to 12 used antibiotics as reflected by inhibition zones. The used antibiotics were Ampicillin and Amoxicillin at a concentration of 10 µg that showed inhibition zones of 6.41 and 6.73 mm, resp., and showed 100% resistance. Cefoxitin at concentration of 30 µg showed inhibition zones of 15.96 mm and 98% resistance. Ten µg concentration of Imipenem showed inhibition zones of 15.92 mm and the strain was 98% resistant. Vancomycin at a concentration of 30 µg, showed average inhibition zones of 11.05 mm and the strain was 95% resistant, Erythromycin, Gentamycin, Streptomycin, and Neomycin with a concentration of 10 µg showed inhibition zones of 15.03, 13.31 14.64 and 16.81 mm resp., and showed 85.4, 86.3, 78.7 mm and 82.6% resistance, resp. Chloramphenicol at a concentration of 30 µg revealed inhibition zone of 16.36 mm and the strains were 78.5% resistant. Ciprofloxacin at a concentration 5 µg showed inhibition zone of 19.85 mm and strains were 56.5% resistant. Tetracycline at a concentration of 30 µg showed area of inhibition of 20.54 mm and the strains were 75.9% resistant as shown in (Table 3).

Table (3): Survey on Antibiotics sensitivity tests conducted against isolated pathogenic UTI *E.coli* species compared with those of (CLSI standard references 2017).

No	Antibiotics Types	Disc code conc (µg)	Σ_{μ} inh zone	Inhibition zone diameter (mm)			Resistance percent %
				Resistant	Intermediate	Susceptible	
1	Ampicillin	AM-10	6.41	11	12 - 13	14	100 %
2	Amoxicillin	AMX-10	6.73	13	14 - 16	17	100 %
3	Cefoxitin	OXSF-30	15.96	14	15 - 17	18	98 %
4	Imipenem	IP-10	15.92	13	14 - 15	16	68 %
5	Vancomycin	VA-30	11.05	9	10 -11	12	95 %
٦	Erythromycin	E-10	15.03	13	14 - 17	18	85.4 %
7	Gentamycin	GM-10	13.31	12	13 - 14	15	86.3 %
8	Streptomycin	S-10	14.64	11	12 - 14	15	78.7 %
9	Neomycin	N-30	16.81	12	13 - 16	17	82.6 %
10	Chloramphenicol	C-30	16.36	12	13 - 17	18	78.5 %
11	Ciprofloxacin	CIP-5	19.85	15	16 - 20	21	56.5 %
12	Tetracycline	Te-30	17.54	14	15 - 18	19	75.9 %

Σ_{μ} inh zones: Summation average of antibiotic effected inhibition zones.

On other side, antibiotic susceptibility tests were conducted against the three strains under study compared with reference strains to determine the susceptibility to antibiotics alone without any other additives. The results revealed that the three isolated UTI *E.coli* were resistant to Ampicillin and amoxicillin as same as reference strains EMG01 and EMG02. The isolates EMG04 and EMG05 were resistant to Cefoxitin, but EMG03 showed inhibition zone diameter (around 14.23 mm) similar to reference strains. All isolates as well as reference strains showed similar inhibition zone diameter (around 15 mm) to Imipenem. The isolate EMG05 was resistant to Vancomycin, but EMG03 and EMG04 was similar to EMG01 and EMG02 in their sensitivity to Vancomycin as showed inhibition zone ranged between 9.17-10.07 mm. The isolates EMG05 was resistant to Gentamycin, but EMG03 and EMG04 showed average inhibition zone of 12.5 mm similar to those of EMG01 and EMG02. All isolates as well as reference strains showed similar inhibition zone diameter (around 14 and 14.7 mm) Erythromycin and Streptomycin, resp. The reference EMG01 was resistant to Neomycin, but the others showed inhibition zones around 16 mm. The reference EMG02 was resistant to Chloramphenicol, but the others showed inh zones around 16 mm. All isolates as well as reference strains were sensitive to Ciprofloxacin and Tetracycline with similar inh zone diameter (around 17-18 mm), resp. Table (4).

4. Chemical characterization of thyme oil

The purified thyme essential oil was conducted using a Trace GC-ISQ mass spectrometric protocol, twenty-six compounds were detected, which were varied in their contents. It was observed that Thymol, P-Cymene and the other ¥ -Terpinene represented the largest percentage of component by 32.3, 21.8 and 13.9%, resp. While Carvacrol, Cineol and Linalool represented the second important percentage of component by 5.1, 4.4 and 3.4%, resp. as shown in Table (5).

Table (4): Antibiotic sensitivity test against the three isolated pathogenic *E.coli* species as estimated by inhibition zones compared with references *E.coli* strains. R: resistant

No	Antibiotic Types	Disc code	*E.coli* strains inhibition zone diameter (mm)				
			EMG01	EMG02	EMG03	EMG04	EMG05
1	Ampicillin	AM 10	R_E	R_E	R_E	R_E	R_E
2	Amoxicillin	AMX 10	R_E	R_E	R_E	R_E	R_E
3	Cefoxitin	OXSF 30	$14.52\pm0.35_B$	$14.12\pm0.25_B$	$14.23\pm0.32_B$	R_B	R_B
4	Imipenem	IMP 10	$15.13\pm0.28_A$	$14.82\pm0.31_B$	$14.94\pm0.37_B$	$14.58\pm0.32_B$	$14.35\pm0.29_B$
5	Vancomycin	VA-K30	$10.07\pm0.16_D$	$9.72\pm0.29_D$	$9.63\pm0.31_D$	$9.16\pm0.41_D$	R_D
6	Erythromycin	E 10	$15.03\pm0.21_A$	$14.81\pm0.33_B$	$14.89\pm0.25_B$	$14.64\pm0.27_B$	$14.29\pm0.36_B$
7	Gentamycin	GM 10	$13.09\pm0.30_C$	$12.77\pm0.24_C$	$12.82\pm0.22_C$	$12.24\pm0.25_C$	R_C
8	Streptomycin	S-10	$14.26\pm0.34_B$	$13.96\pm0.31_B$	$14.15\pm0.19_B$	$13.83\pm0.28_B$	$13.51\pm0.28_B$
9	Neomycin	N 30	R_C	$16.04\pm0.32_C$	$16.39\pm0.32_C$	$16.01\pm0.17_C$	$15.84\pm0.21_C$
10	Chloramphenicol	C -30	$16.57\pm0.26_C$	R_C	$16.23\pm0.41_C$	$16.11\pm0.41_C$	$16.09\pm0.18_C$
11	Ciprofloxacin	CIP-5	$18.53\pm0.21_A$	$18.36\pm0.35_A$	$18.45\pm0.28_A$	$18.23\pm0.35_A$	$18.04\pm0.31_A$
12	Tetracycline	Te-30	$17.09\pm0.34_A$	$16.95\pm0.25_A$	$17.02\pm0.25_A$	$16.65\pm0.23_A$	$16.19\pm0.38_A$

Values represent means ± Standard division (SD) obtained from three treatments.
The means in the same raw or column followed by different letters differ significantly, ($p \geq 0.01$).

Table (5): The chemical composition of the purified thyme essential oil with the retention time (Rt).

No	Compounds	Rt (min)	Ratio (%)
Monoterpenoid phenol			
1	Thymol	31.44	32.2
2	P-Cymene	17.52	21.8
3	Carvacrol	17.52	5.1
4	Methyl carvacrol ether	12.18	1.2
Monoterpenes			
5	¥-Terpinene	19.26	13.9
6	Cineol	21.40	4.4
7	Linalool	37.07	3.4
8	α-Terpinene	7.08	2.4
9	β - Pinene	7.73	2.1
Monoterpenoid ketone			
10	Camphor	10.54	0.4
Monoterpenoids			
11	Camphene	6.28	0.6
Sesquiterpene			
12	β-Caryophyllene	15.43	0.5
Others		--	12.0

On other side, the nano-emulsion was characterized, the droplet size and multiple scattering index (PDI) were measured using the dynamic light scattering (DLS) technique, The obtained Zeta potential analysis from the peaks confirmed that all nanoforms sizes lie between 10 to 100 nanometers. The thyme oil nanoform was lie in two peaks, the main peak average sized by 53,99 ± 18.62 nm, the other peak average sized by 5458 ± 635.9 nm with total zeta average of 67.64 nm the thymol crystal nanoform was more homogenize and lie in one peak by 51,09 ± 21.85 nm with total zeta average of 66.05 nm as shown in Figure (1).

Fig (1): Histogram showing the sizes disruption analysis of purified thyme oil and thymol crystal nanoforms with the specific Zeta averages by nanometer.

5. The antibiotics promoting action by the thyme oil and thymol crystal forms

The effect of different concentrations of thyme oil and thymol crystals either as emulsion or nano emulsion on the growth of isolated UTI *E. coli* as estimated by inhibition zones diameters were shown in Table (6). All used concentrations of thyme oil emulsion (1.5, 2.0 and 2.5%) showed slight inhibition zones around isolated and references strains. The differences in diameters of inhibition zones between 1.5, 2.0 and 2.5% concentrations were insignificant, as showed 6.14, 6.29 and 6.73 mm for isolate EMG03, 6.09, 6.17 and 6.62 mm for EMG04 and 6.05, 6.12 and 6.33 mm for EMG05, resp., compared with reference strains that ranged between 6.16, 6.33 and 6.86 mm for EMG01 and 6.03, 6.12 and 6.23 mm for EMG02, resp. While the use of nano-emulsion of thyme oil caused increased inhibition zones when used against the three *E.coli* isolates with increasing its concentrations. The inhibition zone diameters were 6.89, 6.72 and 6.79 for EMG03; 6.8, 6.51 and 6.61 for EMG04 and were 6.79, 6.46 and 6.55 mm for EMG05 when 1.5, 2.0 and 2.5% concentrations of nano-emulsion were used, resp. The differences in inhibition zone between these concentrations (1.5, 2 and 2.5%) were significant. Although, the inhibition zones of isolated bacteria (EMG03, 04 and 05) were similar to those of reference strain EMG01 and EMG02 (6.93, 8.87 and 10.03 mm) for 1.5, 2.0 and 2.5 concentration of nano emulsion, resp., but they were significantly different from those of reference strain EMG02 which recorded 6.42, 8.56 and 8.93 mm under 1.5, 2.0 and 2.5% thyme oil concentrations (nano-emulsion form), Table (6).

Table (6): Antagonistic effect of different three concentrations of thyme oil and thymol crystal as natural additives on isolated UTI *E.coli sp*. compared with references strains.

Treatment	Type	Conc	*E.coli* sp inhibition zone diameter (mm)				
			EMG01	EMG02	EMG03	EMG04	EMG05
Thyme Oil	Emulsion	1.5%	$6.16\pm0.35_{E}$	$6.03\pm0.26_{E}$	$6.14\pm0.21_{E}$	$6.09\pm0.35_{E}$	$6.05\pm0.28_{E}$
		2.0%	$6.33\pm0.28_{E}$	$6.12\pm0.32_{E}$	$6.29\pm0.27_{E}$	$6.17\pm0.38_{E}$	$6.12\pm0.19_{E}$
		2.5%	$6.86\pm0.31_{E}$	$6.21\pm0.28_{E}$	$6.73\pm0.26_{E}$	$6.62\pm0.35_{E}$	$6.33\pm0.22_{E}$
	Nano emulsion	1.5%	$6.93\pm0.27_{D}$	$6.42\pm0.14_{E}$	$6.89\pm0.22_{D}$	$6.80\pm0.28_{D}$	$6.79\pm0.40_{D}$
		2.0%	$8.87\pm0.34_{B}$	$8.56\pm0.21_{C}$	$8.72\pm0.19_{B}$	$8.51\pm0.24_{B}$	$8.46\pm0.23_{B}$
		2.5%	$10.03\pm0.21_{A}$	$9.82\pm0.35_{A}$	$9.79\pm0.30_{A}$	$9.61\pm0.24_{A}$	$9.55\pm0.29_{A}$
Thymol Crystal	Emulsion	1.5%	$7.09\pm0.32_{D}$	$6.93\pm0.25_{D}$	$7.57\pm0.19_{D}$	$7.29\pm0.30_{D}$	$6.78\pm0.25_{E}$
		2.0%	$7.26\pm0.18_{D}$	$7.15\pm0.29_{D}$	$7.93\pm0.22_{D}$	$7.38\pm0.35_{D}$	$7.14\pm0.31_{D}$
		2.5%	$8.16\pm0.34_{C}$	$7.93\pm0.26_{C}$	$8.09\pm0.31_{B}$	$7.82\pm0.32_{C}$	$7.61\pm0.12_{C}$
	Nano emulsion	1.5%	$8.26\pm0.35_{C}$	$7.93\pm0.20_{C}$	$7.97\pm0.23_{C}$	$7.26\pm0.30_{D}$	$7.22\pm0.44_{D}$
		2.0%	$9.03\pm0.25_{B}$	$8.95\pm0.11_{B}$	$8.90\pm0.37_{B}$	$8.73\pm0.21_{B}$	$8.36\pm0.36_{C}$
		2.5%	$11.09\pm0.39_{A}$	$10.94\pm0.22_{A}$	$10.53\pm0.25_{A}$	$10.32\pm0.30_{A}$	$10.11\pm0.19_{A}$

Values represent means ± Standard division (SD) obtained from three treatments.
The means in the same raw or column followed by different letters differ significantly, ($p \geq 0.01$).

Addition of different concentrations of thymol crystals (emulsion form) against isolated and references strains *E.coli* showed different inhibition zones. The zones were 7.57, 7.73 and 8.09 mm for EMG03; 7.29, 7.38 and 7.82 mm for EMG04 and were 6.78, 7.14 and 761 mm for EMG05 when 1.5, 2.0 and 2.5% concentration of oil crystal (emulsion form) was used, compared with inhibition zones of reference strains that showed 7.09, 7.26 and 8.16 mm for EMG01 and 6.93, 7.15 and 7.93 mm for EMG02, resp. The differences in inhibition zone diameters between used concentrations (1.5, 2.0 and 2.5% oil, crystal emulsion forms) were significant for isolate EMG05. Moreover the use of 2.5% concentrations of crystal (emulsion form) caused significant inhibition zones against all isolated and references *E.coli* compared with those of 1.5 and 2.0% concentrations. In case of use of nano-emulsion form of thymol crystal, the detected inhibition zones under concentrations of 1.5, 2.0 and 2.5% were 7.97, 8.90, 10.53 mm for EMG03; 7.26, 8.73 and 10.32 for EMG04 and 7.22, 8.36 and 10.11 for EMG05, resp., compared with those of reference strains 8.26, 9.03 and 11.09 mm for EMG01 and 7.93, 8.95 and 10.94 mm for EMG02. It was noticeable the significant differences in inhibition zones among the used concentrations of nano thymol crystal (1.5, 2.0 and 2.5%) against either isolated or reference strains. Moreover, uses of 2.5% concentrations of nan-forms of oil or crystal were the most effective among other concentrations as showed a significant inhibition zone against all isolated and reference pathogenic *E.coli*.

On other side, the pathogenic *K. pneumonia* which isolated from UTI patient was resistant to Ampicillin 10 mg and Erythromycin 10 mg, however addition of 2.5% concentration of Emulsion of thyme oil or thymol crystals to ampicillin (AM-10) or Erythromycin (E-10) affected the growth of *K. penumenae* as shown by 6 or 6.2 mm inhibition zone for AM-10 and 16.54 or 18.94 mm inhibition zone for E-10, respectively. This diameter was again increased to 13.02 mm or 15.89 mm resp., after addition of 2.5% nano-emulsion of thyme oil or thymol crystal to AM-10 and increased to 27.08 or 25.02 mm, resp., after addition of E-10. As shown in Table (7).

Table (7): Effect of mixed antibiotics mixed with various forms of thyme oil and thymol crystal (2.5% concentration) against isolated culture of UTI pathogenic *K. pneumoniae* species.

No	Antibiotic Types	DISC CODE	*R_d	Antibiotics culture of UTI *K. pneumoniae* species (inh zone diameter in mm)				
				Bacterial Culture	Emulsion		Nano emulsion	
					Thyme oil	Thymol crystal	Thyme oil	Thymol crystal
1	Ampicillin	AM-10	11	R_E	$6.00\pm0.22_E$	$6.21\pm0.25_E$	$13.02\pm0.21_D$	$15.89\pm0.25_D$
2	Ciprofloxacin	CIP-5	15	$17.91\pm0.15_C$	$19.02\pm0.41_C$	$19.11\pm0.15_C$	$24.38\pm0.26_B$	$25.20\pm0.18_B$
3	Erythromycin	E-10	13	R_C	$16.54\pm0.32_E$	$18.94\pm0.31_C$	$27.08\pm0.21_B$	$29.00\pm0.22_A$
4	Neomycin	N-30	12	$15.58\pm0.20_D$	$17.65\pm0.28_C$	$17.38\pm0.20_C$	$28.68\pm0.29_A$	$29.74\pm0.21_A$
5	Chloramphenicol	C-30	12	$15.89\pm0.37_D$	$17.39\pm0.39_C$	$17.20\pm0.29_C$	$28.43\pm0.41_A$	$29.51\pm0.42_A$

R_d : Resistance to antibiotics when inhibition zones diameters do not exceed these values in mm.
The means in the same raw or column followed by different letters differ significantly, ($p \geq 0.01$).

However, there were significant differences between all oil forms (emulsion and nano-emulsion) in inhibition zone diameters when E-10 was used. The two antibiotics; Neomycin N-30 and Chloramphenicol C-30 showed similar effects when used with emulsion of thyme oil or thymol crystal, as the inhibition zone were 17.38 and 17.19 mm for N-30, and 17.39 and 17.20 mm for C-30, resp. In fact, the nano-emulsion of thyme oil or thymol crystal were similar in their effect, as the inhibition zones were 28.86 and 29.74 mm for N-30, and 28.43 and 29.51 mm for C-30, resp.

It was noticeable that the nano-emulsion of thyme oil or thymol crystal always showed significant differences in inhibition zone diameters than those of emulsion of thyme oil or thymol crystals. Also, *S. saprophyticus* is a gram-positive bacteria isolated from UTI patients and showed resistance to Ampicillin (AM-10) and Ciprofloxacin (CIP-5). However *S. saprophyticus* became sensitive to these antibiotics after addition of emulsion or nano-emulsion of thyme oil or thymol crystal. The inhibition zone detected after addition of emulsion of thyme oil and crystal, separately, were 6.02 and 6.11 mm, for AM-10 and 18.78 and 18.89 mm for CIP-5, resp. as shown in Table (8).

Table (8): Effect of antibiotics mixed with various forms of thyme oil and thymol crystal (2.5% concentration) against isolated UTI pathogenic *S. saprophyticus*.

No	Antibiotic Types	DISC CODE	*R_d	Antibiotics culture of UTI *S. saprophyticus* species (inh zone diameter in mm)				
				Bacterial Culture	Emulsion		Nano emulsion	
					Thyme oil	Thymol crystal	Thyme oil	Thymol crystal
1	Ampicillin	AM-10	11	R_E	$6.02\pm0.18_E$	$6.11\pm0.41_E$	$12.85\pm0.13_D$	$15.62\pm0.25_D$
2	Ciprofloxacin	CIP-5	15	R_E	$18.78\pm0.18_C$	$18.89\pm0.27_C$	$24.07\pm0.25_B$	$25.02\pm0.29_B$
3	Erythromycin	E-10	13	$15.29\pm0.33_D$	$16.26\pm0.17_E$	$18.71\pm0.31_C$	$26.86\pm0.19_B$	$28.87\pm0.29_A$
4	Neomycin	N-30	12	$15.84\pm0.21_D$	$17.38\pm0.35_C$	$17.19\pm0.17_C$	$28.46\pm0.42_A$	$29.51\pm0.27_A$
5	Chloramphenicol	C-30	12	$16.19\pm0.38_D$	$18.31\pm0.37_C$	$19.02\pm0.30_C$	$30.97\pm0.31_A$	$33.96\pm0.17_A$

R_d : Resistance to antibiotics when inhibition zones diameters do not exceed these values in mm.
The means in the same raw or column followed by different letters differ significantly, ($p \geq 0.01$).

The inhibition zones were increased when nano-emulsion of thyme oil or thymol crystal were used, as recorded 12.85 and 15.62 mm for AM-10, and 24.07 and 25.02 mm for CIP-5, resp. The two antibiotics, Neomycin N-30 and Chloramphenicol C-30 affected the growth of *S. saprophyticus* when added with emulsion of thyme oil or crystal, as recorded 15.84 and 17.38 mm for N-30, and 16.19 and 18.31mm for C-30, resp. While in case of nano-emulsion (thyme oil or thymol crystal), the zones were 28.46 and 29.51mm for N-30, and 30.97 and 33.96 mm for C-30, resp., while the use of Erythromycin with emulsion of thyme oil or thymol crystal showed 15.29 and 16.26 mm, and 26.86 and 28.87 mm for nano emulsion of thyme oil or crystal, resp. The nano-emulsion of thyme oil or thymol crystal always showed significant differences in inhibition zone diameters than those of emulsion of thyme oil or thymol crystal. Moreover, significant differences between all oil forms (emulsion and nano-emulsion) in inhibition zone diameters when E-10 was used.

In the same aspect, the determination of the inhibition's zones was dramatically increased by increasing the theme oil and thymol crystal concentrations, it is normal to record the highest concentrations levels of minimal inhibitory concentrations and minimal inhibitory bacterial concentrations (MICs and MBC) which reached to 220 and 440 mg/ml, resp. with bactericidal efficiency (MBC/MIC) by 200% from the bacteriostatic effects. It's clear to noted that, the nano-emulsion forms can sharply decrease the used amounts of thyme oil or thymol crystal to reach the MIC and MBC by 50%. The using of 180 to 220 mg/ml of nanoforms sufficient to reach MBCs for the G^{-ve} bacteria from the UTI *E.coli* and *K, penumenae*. while 145 to 180 mg/ml of nanoforms sufficient to reach MBCs for *S. saprophyticus*, Figure (2).

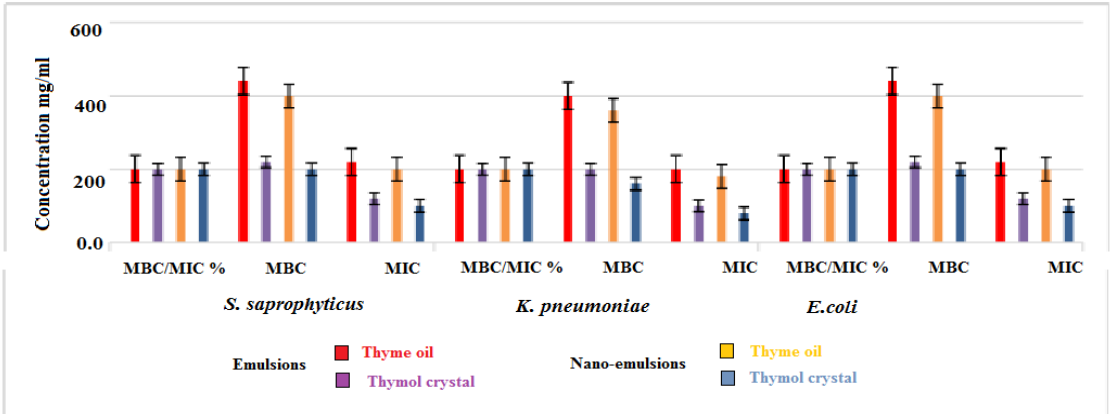

Fig (2): Histogram showing determination of MIC / MBC values for thyme oil and thymol crystal against isolated real cultures of pathogenic UTI *species*.

DISCUSSION

The emergence of resistance to different classes of antibiotics in previously susceptible bacterial pathogens is a major challenge to infectious disease. Thus, it is important that forcefully implement strategies to reduce the rate of appearance and spread of resistant bacteria to catch up with bacterial resistance development Abdelmonem *et al.*, (2020). While, bacteria are considered the main and important causes of these infections, and the bacteria that cause UTI vary between the widespread bacteria, the most common are Grame negative (G^{-ve}) bacteria and other genera according to the investigation of (Haghi, 2004). The results of this study showed that *E.coli* bacteria is one of the most members of the enteric family that causes about 81.4% of urinary tract infections followed by *Klebsiella pneumonia* about 10.26 %. This is consistent with those obtained by (Azab, 2021) and Jawetz *et al.*, (2016), they reported that *E.coli* have been found to be the most common causative

organism of UTI in many countries followed by *K. pneumonia* and even globally, *E.coli* is the most common pathogen causing UTIs, it causes 80–85% of cases. Three G^{-ve} *E.coli* species were selected from the three infection levels mentioned in Table (1). They named; EMG03 which represent the minor infection level, EMG04 represents the average or moderate level of infection and EMG05, which represents the severe level of infection. Two reference multi drug resistant strains were used from the National Research Center: EMG01 is resistant to Ampicillin, Neomycin, and EMG02 is resistant to Ampicillin and Chloramphenicol which recently designed as models designed by (Fahim and Hussein, 2017). The UTI isolates of this study differed in their resistance to the 12 used antibiotics with percentages between 56-100%.The *E.coli* isolates were 100% resistant to used beta-lactams and 56% for fluoroquinolones as shown in (Table 3). In this regard, Sumon *et al.,* (2023) reported that *E.coli* strains are UTI bacteria that can generate a large spectrum of beta-lactam enzymes, making them resistant to most beta-lactam antibiotics. The results of this study also showed that *K. pneumonia* was the second cause of UTIs with percentage of 10.2% and was 100% resistance to ampicillin and Amoxicillin (beta-lactam antibiotics). This is because it possesses a penicillinase codified in its genome Sutaria *et al.*, (2018), and its ability to acquire multiple mobile plasmids by horizontal transfer Wyres and Holt (2018). The present study detected *S. saprophyticus* with 2% in urine samples which were resistant to beta-lactam antibiotics and Ciprofloxacin (Tables 2 and 8). *S. saprophyticus* are the most common and most important among Staphylococcal species associated with UTIs Ferreira-Grosso *et al.*, (2017). They also found that fifteen out of 22 (68.2%) of *S. saprophyticus* isolates were resistant to Penicillin by the E-test and 19 out of 22 carried the *bla*Z gene that encodes production of a penicillinase which inactivates Penicillin by hydrolysis of the beta-lactam ring. The increase in resistance to almost all classes of antibiotics cause difficulty in treating an infection. Developing new efficient therapies or complementing existing treatments is a priority, and essential oils (EOs) provide an alternative. EOs could act as antibiotic adjuvant and enhance or facilitate antibiotic activity Castillo *et al.*, (2023). In this study, thyme oil was used as natural additive to the support antibiotic action against UTI isolated bacteria. While, the other forms (emulsion and nano-emulsion) of thyme oil and thymol crystals, especially nano-emulsion of thymol crystals were more effective against the three selected UTI *E.coli* isolates and the two standard ones. As well as, against *K. pneumonia* and *S. saprophyticus* when used alone (Table 2) or added with used antibiotics (Table 7). The nano-form sizes prepared in this study lies between 10 to 100 nanometers as shown in figs (3 and 4), which is consistent with those of Sugumar *et al*., (2014). This nano-emulsion of essential oils (EOs) has high entrapment efficiency, small particle size and stability. However, crude hydrated extract thyme oil form did not show high antagonistic activity against isolated pathogens, this may be due to the particular impermeability of the external membrane of bacteria to essential oil along with efflux mechanism Longbottom *et al.,* (2004). The composition of thyme EO resulted in the present study was comparable with the composition previously reported Mariana Romo-Castillo *et al.*, (2023) as thymol, cymene, terpinene, carvacrol, cineol and linalool were the main component detected in this study. Apparently, the antimicrobial activity of the EO analyzed is related to the presence of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene) Rota *et al*., (2008). In this study, use of 2.5% concentration of nano-emulsion of thymol crystal (compared with other used forms of oil), without antibiotic, showed significant inhibition zone against all isolated and reference pathogenic *E.coli*. The nano-emulsion of thymol crystal also can sharply reduce the used amounts of thyme oil or thymol crystal to reach the MIC and MBC by 50% as shown in (Fig 2). However, when nano-emulsion of thymol crystal was combined with each of the 5 used antibiotics and added against UTI G^{-ve} *E.coli*, *K. penumenae* and G^{+ve} *S. saprophyticus*, the inhibition zone diameters were significantly increased, moreover some *E.coli* (EMG03, 04 and 05) isolates that

were resistant to beta-lactams, aminoglycosides and glycolipids antibiotics get affected and become susceptible. The efficacy of nano-emulsion of thymol crystal was the highest compared with the aqueous emulsion of either thyme oil, or the aqueous emulsion of thymol crystal. This is because it possesses many medicinal properties including antioxidant, free radical removal, and antibacterial components. These results are consistent with those obtained by Al-Shalabi *et al*., (2022). The most likely mechanism of action of thymol is its ability to alter cell membranes Chauhan and Kang (2014). On the one hand, the hydrophilic part of the molecule interacts with the polar part of the bacterial cell membrane while the hydrophobic benzene ring and lipid side chains of thymol interact with the hydrophobic part of phospholipids, causing a loss of membrane stability and alterations in its permeability Li *et al.,* (2022). Although this seems to be the main mechanism, other studies specify that it may also have internal targets and interact at the mitochondrial level, causing the disruption of adenosine triphosphate (ATP) synthesis and inducing the generation of Na^{+} and Ca^{+2} metabolic disturbances, leading to an excess of oxygen free radicals that cause cell death Cheng *et al.,* (2020). In this study, the transmission electron microscope image of *E.coli* isolate treated with 2.5% of thymol crystal confirmed the revelation disruption and phenotypic changes of cells compared with untreated control (Fig 3), this allows antibiotics to penetrate and get into the cell and restore their actions, and therefore help in bacterial death.

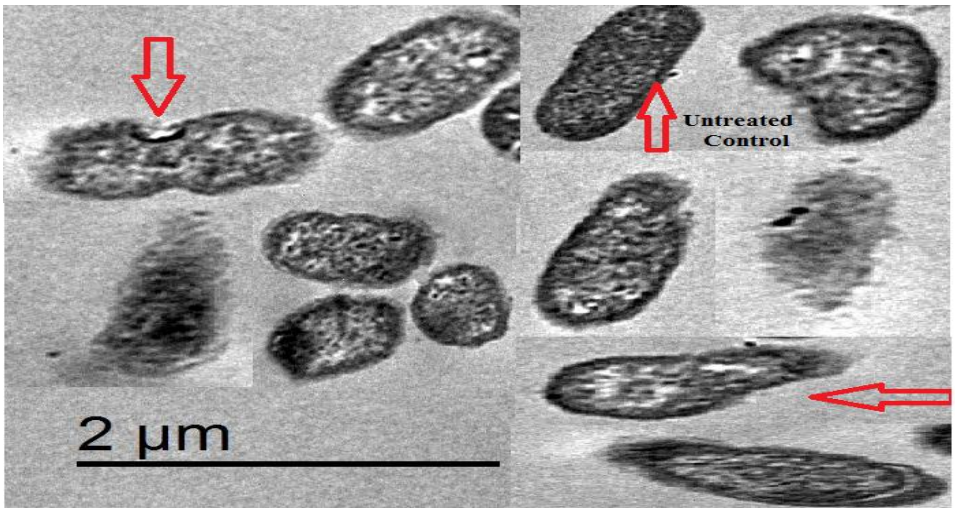

Fig. (3): TEM image of pathogenic UTI *E.coli* treated with 2.5% of thymol crystal nanoform.

The results of TEM image of this study were consistent with SEM image obtained by Sara Garcia-Salinas *et al*., (2018) they studied the effect of most active compounds found of thyme oil on *E.coli* and found that untreated cells of *E.coli* appeared with normal rod shaped, regular, and with intact morphology in contrast to MIC-treated cells. Also, the SEM images showed morphological alterations and lyses of the outer membrane integrity in cells exposed at MICs of thyme oil active compounds. In this context, Cuaron *et al.,* (2023) reported that the reason for the numerous synergistic effects that emerged both in the present study and in other investigations could be linked to the mechanism of action of EOs, in particular their ability to interact with the permeability of the bacterial cell in G^{-ve} bacteria and their capability to alter the gene regulation involved in the cell wall metabolism in G^{+ve} bacteria Rios and Recio (2005). The interaction of EOs with the bacterial cell wall could be used to improve the activity of antibiotics, facilitating their penetration with a reduction of therapeutic doses Bubonja-Sonje *et al.,* (2011). The increased zones of inhibition are consistent with some evidence that components

of EOs interact synergistically with antibiotics by interfering with antibiotic resistance mechanisms. Inhibiting antibiotic resistance mechanisms has already been successfully used in antibiotic chemotherapy. For example, clavulanic acid inhibits γ-lactamases and is commonly used to increase the activity of Ampicillin, according to Van Vuuren and Viljoen (2011) as Ampicillin belongs to the group of beta-lactamases. In this respect, the mechanism of action of synergistic effect of antibiotics with essential oils was proposed by Castillo *et al.,* (2023) they explained that essential oils alter the hyper-muco-viscosity phenotype of the strains, breaking the lipid-soluble barrier that prevents the internalization and action of antibiotics. Once these factors are modified, antibiotics and essential oils can enter the pathogen and induce its death. Thyme oil is one member of essential oils labeled as GRAS (generally recognized as safe) and listed in the natural additive's preservatives admitted in the European Union Register of Feed Additives, which establishes authorized feed additives in the European market Kuate (2017). EOs encapsulated into nanoparticles or incorporated in edible/biodegradable coatings, is a possible solution to improve antibiotic actions against resistant bacteria and also improve food preservation El-Zehery *et al.*, (2022).

Conclusion

The study concluded that adding a nano emulsion of thyme oil or thymol crystal at a concentration of 2.5% to antibiotic treatment for bacteria causing urinary tract infections increases the antibiotics effectiveness, strengthens their action, and reduces bacterial resistance to antibiotics, which minimize the doses used and also reduces the duration of treatment and lowing the economic cost.

Recommendations

Firstly, we recommend the using the active compound thymol in nanometers form at a concentration of 2.5% to support antibiotics.

Secondly, We also recommend making an aqueous emulsification in the nanometric form of both the crude oil and the pure active ingredient.

Finally, Including the thyme oil in therapeutic nutrition programs due to its important composition of thymol, carvacrol, and other antibacterial phenolic component against the invasion antibiotic-resistant pathogenic bacteria.

Conflicts of Interest

The authors declare no conflict of interest.

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تعزيز عمل المضادات الحيوية باستخدام أشكال زيت الزعتر ضد البكتيريا المقاومة المسببة لعدوي المسالك البولية

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الملخص العربى

في العقود الماضية، معدل تزايد مقاومة المضادات الحيوية كان عائقًا حاسمًا أمام نجاح علاج العديد من الأمراض البكتيرية. ونظرا لتزايد هذه المقاومة خاصة في مسببات التهابات المسالك البولية (UTI)، وجب دراسة أستراتيجيات بديلة للعلاج. ففي العمل الحالي، تم عزل أربع أنواع بكتيرية رئيسية من المرضى المصابين بالتهاب المسالك البولية. حيث تم توصيف العزلات بيوكيميائياً بأنها بكتيريا الأيشرشيا كولاي والكليبسيلا نومونيا والأستافيلوكوكس سابروفيتكس كذلك الأنتيروباكتر فيكالس. واختلفت عزلات التهاب المسالك البولية هذه في مقاومتها للمضادات الحيوية الأثنى عشر المستخدمة بنسب تتراوح بين ٥٦ – ١٠٠ % وبالتالي لا يمكن استخدامها للعلاج التجريبي في العديد من المناطق الجغرافية. كما أظهر محتوي زيت الزعتر على بعض المركبات النشطة مثل الثيمول والبيتا سيمين والكارفاكرول والسينول واللينالول بنسب مختلفة بعد فحصها بجهاز الفصل الكروماتوجرافي الغازي للطيف الكتلي GC-ISQ. كذلك أظهرت إضافة زيت الزعتر وبلورات الثيمول على أشكال مستحلبات ومستحلبات نانومتريه بتركيز (٥,٢ %) إلى المضادات الحيوية المستخدمة ضد العزلات المقاومة لالتهاب المسالك البولية تأثيراً تآزرياً ضد عزلات الإيشريشيا القولونية التي أظهرت مقاومة للمضادات الحيوية من الأنواع بيتا لاكتام، أمينوجليكوزيدات وجليكوليبيدات. حيث بدأت في التأثر الى أن تصبح حساسة للمضادات الحيوية المستخدمة. علاوة على ذلك، تم تقليل كميات زيت الزعتر أو بلورات الثيمول المستخدمة للوصول إلى أقل تركيز مثبط أو قاتل MIC وMBC بنسبة ٥٠ % عند تطبيق الأشكال النانوميترية لأي من الصورتين. في حين كشفت صورة المجهر الإلكتروني النافذ (TEM) لعزلة الإيشريشيا القولونية المعالجة ببلورات الثيمول النانوميترية بتركيز (٥,٢ %) عن خلل وتغيرات مظهرية في جدار الخلية مقارنة مع الخلايا الغير معالجة، وبالتالي تحسين العمل التآزري من خلال زيادة اختراق ونشاط المضادات الحيوية المستخدمة.