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THYME AND CUMIN NANOEMULSION AS A PROMISING ANTIMICROBIAL AGENT AGAINST MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS*

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This study aimed to compare thyme, cumin extracts, and their nanoemulsion as antibacterial on S.aureus. The methods include , preparation of aqueous, oil extract and nanoemulsion from thyme and cumin, determination of MIC using the resazurin microdilution method, detection of mecA and coagulase genes by PCR before and after the treatment with nanoemulsions, figuring the bacteria after treatment using TEM. the result showed that the MIC values of thymus oil 100% , thymus oil nano emulsion 20% , cumin oil 100% ,cumin oil nanoemulsion 20% ,mix oil 20% ,thymus oil 20% ,cumin oil 20% (0.048, 0.156 , 1.562 ,10 , 0.625 , 0.312 , 10 ,) mg/m respectively , mecA and coagulase genes was absent after the in vitro treatment and TEM microscope showed the bacteria with extensive damage to the cell membrane. the result showed that nanoemulsion has a higher effect on S. aureus than essential oil alone

***Keywords**: Thymus, cumin, nanoparticle, antibacterial activity, TEM microscope*

INTRODUCTION

Staphylococcus is widely disseminated in water, soil and the air, in addition to its harmful infection of numerous animal species and poultry[1]. In addition to causing a wide range of infections in the hospital and community[2].

Staphylococcus aureus is recognized worldwide as a significant food-borne pathogen, and it is a major factor in instances of food poisoning and outbreaks[3]. Methicillin-resistant *S. aureus* isolates are primarily from poultry and human samples, and they contain numerous antimicrobial resistance genes such as penicillin, tetracycline, and clindamycin, as well as mecA, which was detected by PCR[4] *Staphylococcus aureus* causes undesirably infections and facilely acquires antibiotics resistance. Even antibiotic-sensible S. aureus can remain alive with antibiotic therapy and tolerate high antibiotic concentrations necessitating prolonged treatment and surgical procedures[5]. Essential oils are oily liquids that are volatile, clear, and rarely colored. They are lipid-soluble inorganic solvents with antiviral, antimicrobial, insecticidal, antioxidant properties, and antifungal properties[6-8].

More than (400) medical plants are used in various countries in the world between them, cumin has been applied as a spice ago ancient times and is a very common spice universal due to its unique aroma .whereas, thyme and rosemary are commonly used and their oils possess the main antioxidant and antimicrobial[9-11]. The antimicrobial action of the cumin oil were discovered through the limitation of minimal inhibitory concentrations (MIC). They found that *Staphylococcus aureus*

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and Listeria *amonocytogenes* had MIC values of 1 and 2 µ l/ml,[12].

Thymol one of the main components of thyme essential oil has been used in traditional medicine as an antibacterial, antiviral, and antiseptic agent[13]. The effect of cumin essential oil (CEO) nanoemulsion was shown to have significant antimicrobial activity against various pathogens such as (*Staphylococcus aureus*, and *Salmonella Typhimurium*)[14].

Nanobiotechnology is now widely used in medicine and agriculture. Many diseases that cannot be treated today may be treated in the future thanks to nanotechnology. The employment of nanotechnology in medical therapeutics necessitates a thorough assessment of its danger and safety factors. Scientists who oppose the utilization of nanotechnology correspond that advancement in this field must continue because it troth great benefits, but testing must be done to ensure its safety in humans. Nano emulsions are used to enhance the action of essential oils by increasing their dissolution rate in the media, minimizing the impact on the product's performance criteria, and increasing their antimicrobial action. Investigating many nan emulsions that affect the deposition of EOs to the cell membrane and the antimicrobial action process will aid in the development of more efficient distribution systems and the use of EOs in real-world food systems[15]. The disadvantages of essential oil quantity dosing can be countered by constructing those as micro- and nan emulsions. These emulsions hold the extra feature of becoming nanometer in size, letting them be used as an efficient medication delivery system due to their thermodynamic properties[16].

The aim objective of the study is to find alternatives to chemical antibiotics to which microbes have become resistant, and these alternatives can be used in the treatment of humans and animals with low concentrations and with greater benefit because they do not have side effects that may lead to poisoning and killing beneficial microbes in the human body that benefit humans.

It can also be used to preserve food, and because these materials have a strong taste and smell, the very low concentration, especially in the case of nano-emulsions, makes the taste and smell very little or non-existent, and thus we preserve the food without any effect that may be caused by industrial preservatives.

Also, we have a modern synergy by mixing two nanoemulsion (cumin and thyme), the aim of which is to obtain a mixture with a stronger effect with a lower concentration of each substance. This is the beginning of more advanced studies and research to make various new materials from natural materials that have no side effect on humans.

MATERIALS AND METHODS

Preparation of *Staphylococcus aureus* strain:

This study was approved by the Institutional Animals Care and Use Committee, Research Ethics Board, Faculty of Veterinary Medicine, Benha University (No. BUFVTM 34-10-22) following animal welfare guidelines.

Field isolates that were confirmed by PCR as *Staphylococcus aureus* and were gained from Animal Health Research Institute, Egypt .

S. aureus isolation and identification were done according to BAM: 2001 and ISO 6888-3:2003(en). Only 5 suspected colonies were picked up and then inoculated into test tubes containing 5 ml of sterile brain heart infusion broth then incubated at 37°C /24 hrs for further confirmation based on Gram staining, catalase test, and coagulase test[17].

preparation of thyme and cumin aqueous and oil extracts

The aqueous extracts were prepared using the decoction method reported by (18) with a few modifications

A 250 ml flask containing 20 g of each dried spice and 100 ml of sterile distilled water was used to create the water extract.After giving the mixture a good stir, it was allowed to stand for 24 hours at a temperature of 4°, to obtain the extract boiling mixture for 15 minutes is necessary. After being run through a muslin cloth, the supernatant was centrifuged (30000g, 15 min).

Cumin (*Cuminum cyminum*) and thyme (*Thymus vulgaris*) oil extracts were obtained at National Research center (NRC). Tween (80) was obtained from the (Sigma-Aldrich Co). Double-distilled and deionized water was filtered before use.

preparation and characterization of nanoemulsion

According to[19], which was prepared in the Nanomaterials Research and Synthesis Unit, oils (20 ml) from thyme or cumin oils or mix (each oil was 10 ml), distilled deionized water (50 ml), and Tween 80 (30 ml), were mixed for half an hour in a homogeneous blender 1500 watts. Then, distilled water was slowly added to the mixed oil phase.And this route used to prepare nanoemulsions from aqueous extracts

To characterise the nanoemulsion and measure electrical conductivity, viscosity , zeta potential (surface charge) by the Zeta device NANOTRAC-WAVE II Zetasizer (MICROTRAC, USA) and to determine the size of the droplet and the distribution of the microemulsion (polydispersity indexes PDI), transmission electron microscopy (TEM) monitoring is used (JEM (1400F) HRTEM) at ray power of (300) keV . acc ording to[20].

Gas chromatography-mass spectrometry (GC-MS) was used by Nawah Scientific Inc. to analyse oils and nanoemulsion components. Analysis of the mass spectrum was used to confirm this. using the 1GC/MS-MS database from the National Institute of Standard and Technology (NIST), which comprises more than (62,000) styles and a library with a collection of unidentified component spectra. The names, molecular weights, and structures of the components of the test materials were established..according to[21].

The Vero Green Monkey cell stripe was given to [Nawah Scientific Inc] for cell culture in [Egypt, Cairo, Mokatam], where cells were raised in DMEM Dulbecco's Modified Eagle Medium mix with (100 mg/ml) streptomycin, (100 unit/ml) penicillin and (10%). foetal cow serum that has been heated in a 37 °C environment with a 5% (v/v) CO2 humidity. Additionally, . cell viability was assessed using the (SRB) sulforhodamine B analyse at various concentrations (0.01, 0.1, 1, 10, 100 ug/ml).as stated in the report by[22].

Molecular analyses

DNA was extracted from *S. aureus* isolates and was tested for the presence of *mec*A and coagulase genes DNA extraction was done using Quick-DNA Miniprep Plus Kit from - Cat No. D4068. Oligonucleotide Primers were supplied from Metabion (Germany).

200 µl of sample suspension were treated at 560 °C for 10 min. with 10 µl of proteinase K and 200 µl of lysis buffer. The lysate was then given 200 µl of 100% ethanol. The samples were then centrifuged and washed. With the help of 100 µl of elution buffer, DNA was extracted.

Table (1) below lists the primers that were given by Metabion (Germany). A 25µl reaction including 12.5 µl of Emerald AMP Max PCR master mix (Takara, Japan), 6 µl of DNA template, 1 µl of each primer at a concentration of 20 pmol, and 4.5 µl of water was utilised to employ the primers.

The product of PCR was separated via gel electrophoresis using 1.5% agarose gel (Applichem, Germany, GmbH) stained with Ethidium bromide and photographed with a gel documentation system under ultraviolet light.

Resazurin microtiter test for MIC measurement of antibacterial activity (REMA)

the REMA method was employed[25]. Resazurin was created by dissolving 0.015 g, vortexing it, sterilising it with 0.22 lm filters. The 96-well plate got 100 µl of MHB broth for each well. Each aqueous extract's couple combinations and 100 µl of their stock solution at a ratio of 1:1 were then added. 100 to 0.781 mg/ml concentration for each extract resulted from the two-fold dilutions that carried out on the plate columns . Each bacterial inoculum, containing 100 µl, was added to each well at a concentration of 1-3 105 CFU/ml.24 hrs at 37 °C were spent incubating the plates.

Following incubation, then 30 µl of resazurin solution were added to each well and re-incubation of the plates for a further 1-2 hrs.

Table 1: Primers sequences of targeted genes in *S.areus.*

Target gene	**Nucleotide sequence 5`-3`**	Amplified segment size	Reference
***mec*A**	F AGAAGATGGTATGTGGAAGTTAG	**583**	23
	R ATGTATGTGCGATTGTATTGC		
Co-agulase	F ACCACAAGGTACTGAATCAACG	**600-1000**	24

Transmission Electron Microscopy (TEM)

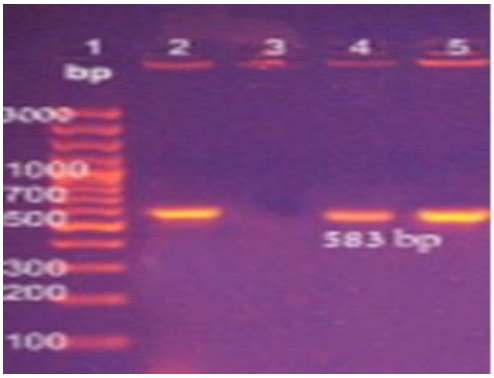
The effect of the chemical on bacterial cell morphology caused by the interaction between nanoemulsion and S. aureus was assessed by a 1400F HRTEM JEM and a beam energy with 300 KV.

RESULTS AND DISCUSSION

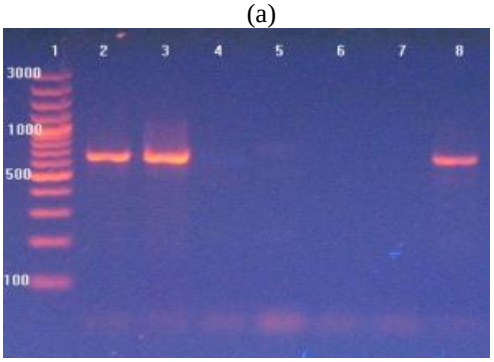
Results

Molecular confirmation of *S. aureus*

In this approach, PCR was used to detect the *mec*A gene and coagulase (coa) genes in *S. aureus* isolates. 2/5 isolates produced a single band to coa on PCR, with molecular sizes ranging from 600 to 1000 bp, while the *mec*A gene detects 583 bp in two isolates Fig. (1).



(a)



(b)

Fig. 1: a. Molecular diagnosis of *S. aureus* by PCR procedure based on mecA gene sequences showing positive bands at 583 bp., Lane 1: DNA Ladder, Lane 2: control positive, Lane 3: control Negative and Lane 4-5: samples.

b. Molecular diagnosis of *S. aureus by* PCR procedure based on coa gene sequences, Lane 1: DNA Ladder, Lane 2-6: samples, Lane 7: control Negative, and Lane 8: control positive.

Description of nano-emulsions

The TEM findings showed that the nano-emulsions of each oil measured as following Thymus oil 51.3 ±1.40 nm, Cumin oil 28.61± 0.56 nm, and mix oil 17.78± 0.82 nm. As seen in Fig. (2), there is no aggregation, spherical nature and uniformity in size.

The conductivity, viscosity, polydispersity index (PDI), and zeta potentials of thyme oil and cumin oil nano-emulsion (20% oil, in water) and combined (10% thyme oil + 10% cumin oil) (Oil/water) were tested, and they were, respectively, 0.185 ms/cm, 0.8872, 0.368, and -5.68 mV ±3.99. Cumin oil nano-emulsion measurements are as follows: 0.187 ms/cm, 0.8872, 0.232, and -5.57 mV± 3.41.Specifically, mixed oil nano-emulsion had values of 0.136 ms/cm, 0.8872, 0.2, and -1.16 mV ±12.05. Fig. (2 a,b,c).

The SRB assay was used to determine cell viability.Aliquots of a 100 L cell suspension (5x103 cells) were incubated in full medium for 24 hours in 96-well plates. Another aliquot of 100 L of medium containing different doses of medicines was then used to treat the cells. 150 L of 10% TCA was added to the media after 72 hours, and it was then incubated at 4 °C for 1 hour. The cells were rinsed with distilled water five times after the TCA solution was removed. Aliquots of 0.4% w/v SRB solution in 70 L were added and incubated for 10 minutes at room temperature in the dark. Plates were subjected to three 1% acetic acid washings before being left to air dry.

After that, 150 L of TRIS (10 mM) was used to dissolve the protein-bound SRB stain, and a BMG LABTECH®- FLUOstar Omega microplate reader was used to detect the absorbance at 540 nm (Ortenberg, Germany). The viability percentage of thyme nano-emulsions at various concentrations was 97.340, 97.210, 95.42., 94.80, and 98.56 after 72 hours of incubation. Additionally, mix nanoemulsions were 96.31, 99.210, 97.42, and 80.92 whereas cumin nanoemulsions were 99.59, 97.210, 95.42 and 89.399. This means that the IC50 was greater than 100 ug/ml, as seen in Fig. (3).

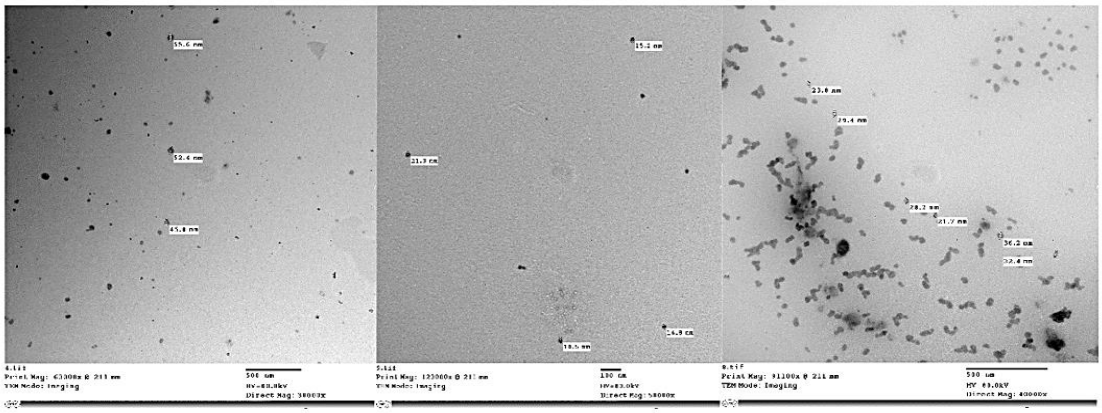


Fig. 2: TEM **a.** Thyme nanoemulsion, **b.** thyme-cumin mix nano emulsion ,**c.** Cumin nanoemulsion revealed that droplets size of Thymus oil, Cumin oil and mix oil nano-emulsion which measured the 51.3 ±1.40 nm, 28.61 ±0.56 nm,17.78 ±0.82 nm, respectively.

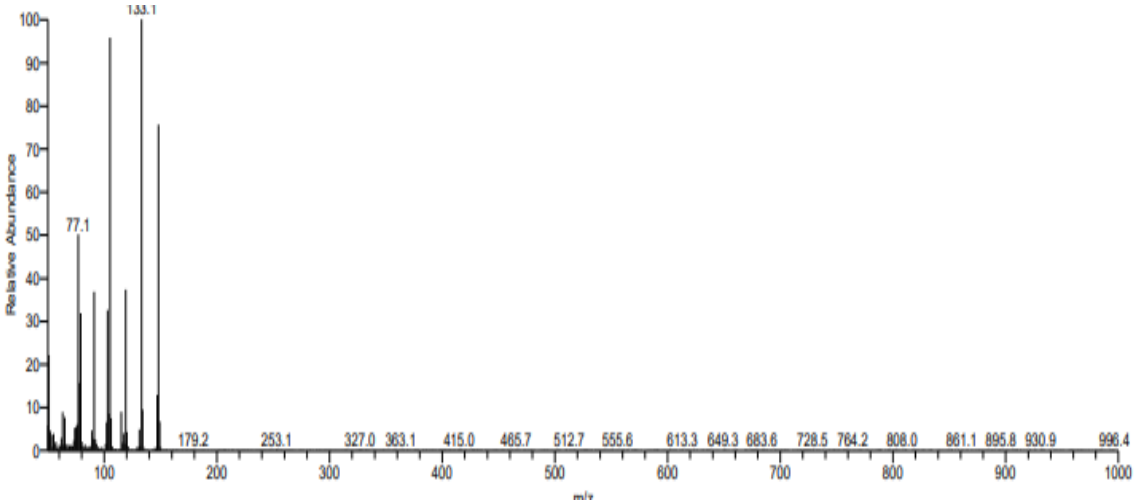


Fig3. a : Mass Spectra Profile of GC-MS analysis of thyme oil.

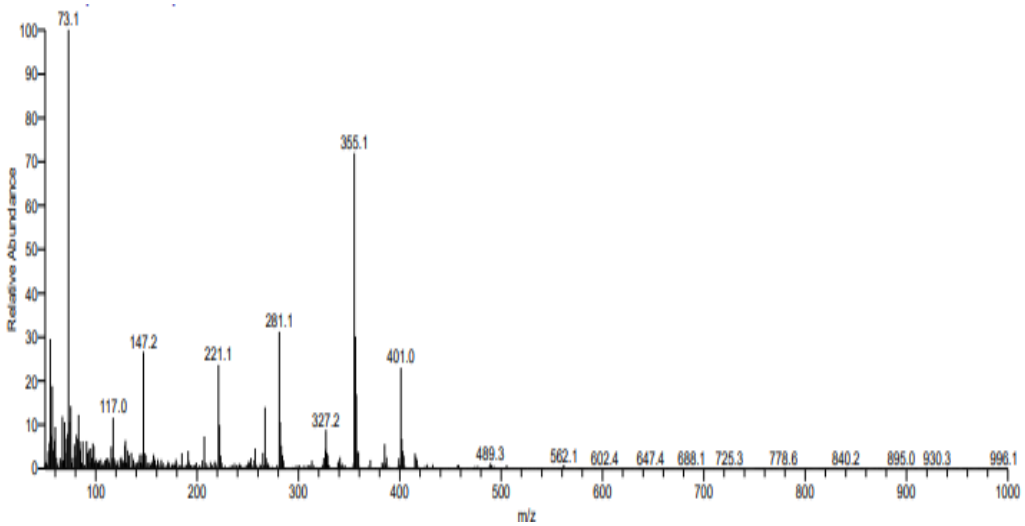


Fig3. b: Mass Spectra Profile of GC-MS analysis of thyme nano-emulsion.

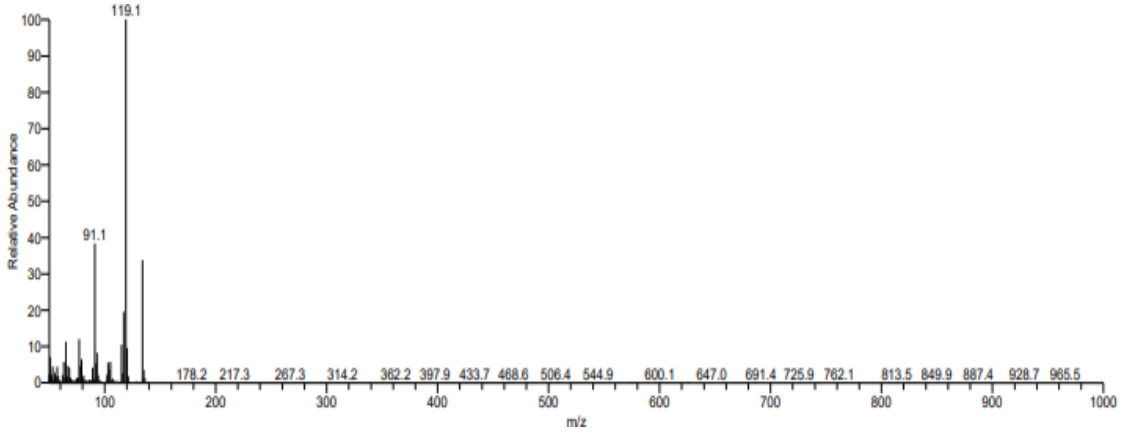


Fig3. c: Mass Spectra Profile of GC-MS analysis of cumin oil.

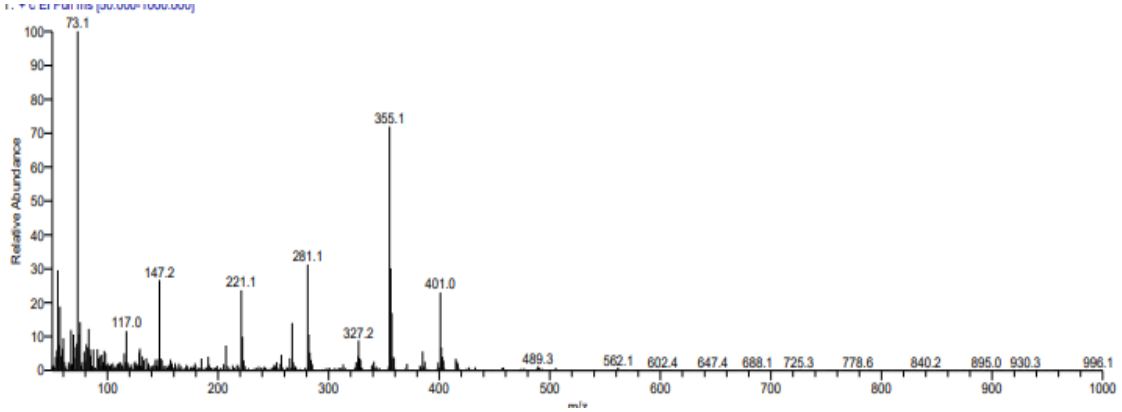


Fig 3.d: Mass Spectra Profile of GC-MS analysis of cumin nano-emulsion.

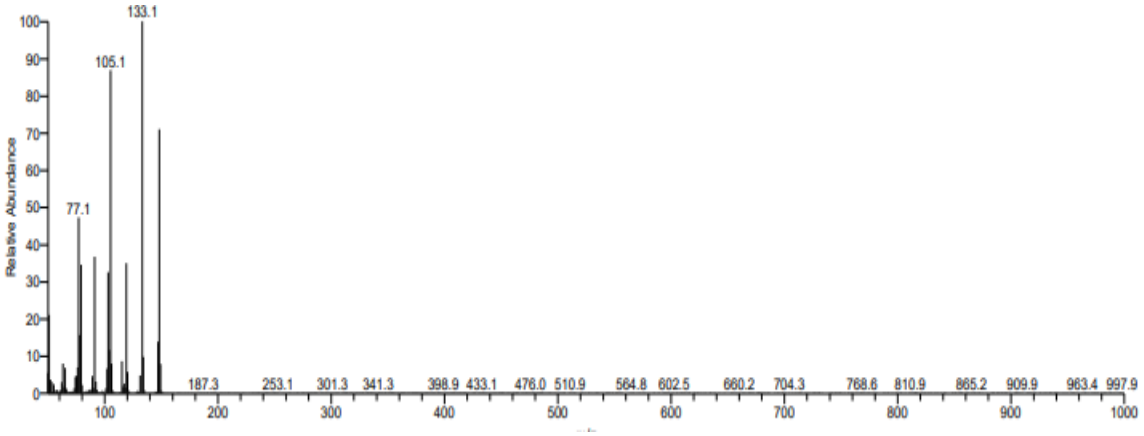


Fig 3.e: Mass Spectra Profile of GC-MS analysis of mix (10 % thyme +10% cumin) nano-emulsion.

Chemical composition of oils and their mix nanoemulsion

The chemical makeup of the three nanoemulsions is shown in **Table (2)**. Thyme oil contained nine chemical components, however, the 20% thyme nanoemulsion had just seven, as indicated in the **Fig. (3 a,b)**. Cumin oil was made up of nine chemical components, whereas 20% cumin nanoemulsion only comprised eight, as indicated in the **Fig. (3 c, d)** and the mixture of (10% thyme + 10% cumin) nanoemulsion eight chemical components As shown in the **Fig., (3 e)**.

In Vitro detection of antibacterial activity of plant extracts nanoemulsion

The diameter of the inhibitory zone of bacterial growth surrounding the disc was measured to determine the antibacterial activity of plant extracts and nanoemulsion against the *S.aureus* strain, as shown in **table (2).**

Table 2: Chemical structure of thyme cumin and mixed nanoemulsion.

Thyme oil		20% thyme nanoemulsion		Cumin oil		20% cumin nanoemulsion		10 % thyme +10% cumin	
Compound	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**
O-cymene	4.98	Octadecenoic acid	1.13	2-methyl-3-phenyl	43.78	Oleyl palmitoleate	12.44	2,5-Dimethoxy-p-cymene	7.32
Decenal	1.55	Ethanaminium	2.95	cymene	6.47	9-Hexadecenoic Acid	30.53	Anthole (anise camphor)	17.06
Dodecadienal	1.33	2-Hydroxy-3-[(9E)-9-OCtadec	23.36	C-terpene	6.7	Cetyl linoleate	8.74	Hexadecenoic Acid	19.91
Phenol	24.68	Glycidyl locate	5.63	Linolsaeure	8.31	anise camphor	3.48	9- Behenyl palmitoleate	31.54
Palmiticaacid	24.24	Enology	27.44	Estragola	4.34	Dodecadienal	1.33	Thymol	2.97
Octade cadienoic acid	11.54	1,2,3propanetriylester	32.94	2-Caren-10-al	7.19	pyrrolidin	1.55	Oleyl palmitoleate	11.87
Trimethylsiy l	26.07	oleic acid	1.30	Palmitic Acid	14.06	oleyl oleate	33.19	1,3-Diolein	5.7
Glycidyl Oleate	1.62			Eugenol	5.37	1,3-Diolein	2.3	palmitoleate	31.54
Enoyloxy	1.26			Linoelaidic acid,trimethylsilyl ester	6.79				

The disc diffusion method is used to determine the doses and concentrations of thyme oil, cumin oil, and nanoemulsion in *S.aureus* strains.

MIC also was detected using Resazurin microtiter assay, and resulted in MIC was placed in the **table (3).**

The result showed that thyme oil nanoemulsion in a concentration of 20% has a MIC of 0.156 µg/ml which was higher than the MIC of thyme oil alone even in the concentration of 100%. Cumin oil nanoemulsion showed also higher MIC than Cumin oil. Mix thyme, and cumin oil nanoemulsion,also resulted in higher MIC in comparison with other oils alone.

Table 3: Antimicrobial activity of nano emulsion on 3 staphylococcus aureus strains.

NO	Material	Concentration	*S. aureus* 1	*S. aureus* 2	*S. aureus* 3	MIC
1	Thyme oil	100%	2	2.5	1.5	0.048 µg/ml
2	Thyme oil nanoemulsion	20%	1.5	1.5	1.0	0.156 µg/ml
3	Cumin oil	100%	1	1	1	1.562 µg/ml
4	Cumin oil nanoemulsion	20%	0.5	0.3	_	10 µg/ml
5	Mix thymus,Cumin oil nano emulsion	20%	1	0.5	0.5	0.625 µg/ml
6	Thyme oil	20%	1.5	0.7	0.8	0.312 µg/ml
7	Cumin oil	20%	1	1.3	1.5	10 µg/ml
8	Cumin aqueous extract	20%	1.5	1.2	1.5	20 µg/ml
9	Cumin aqueous extract	100%	1	1.2	1	50 µg/ml
10	Nano cumin aqueous extract	20%	0.7	0.8	0.9	10 µg/ml

Results of PCR tests to identify the coagulase and mecA genes in S. aureus following various treatments

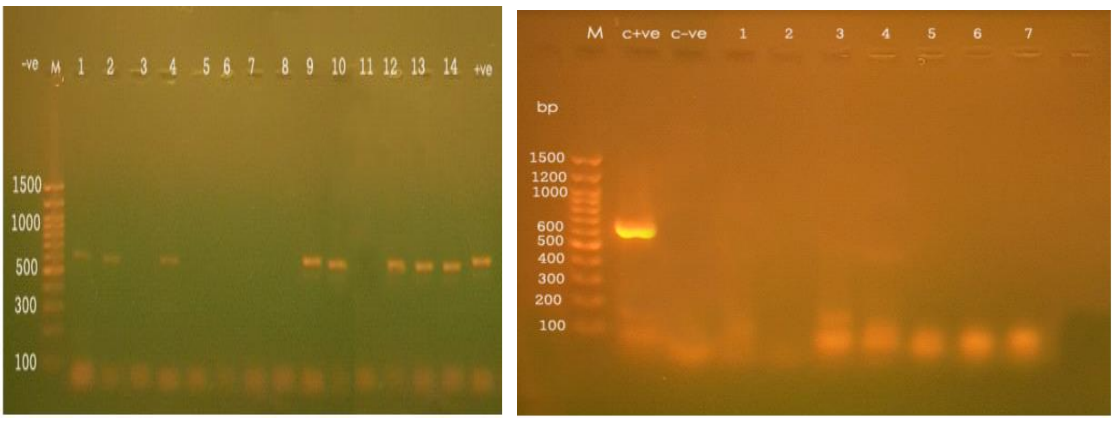
It was displayed in **table (4 and 5)** that larger effect of the chemicals on the coagulase gene than the mecA geneafter being administered to the bacteria showing the effect of treatment on both genes **Fig. (4. a and b)**

Table 4: Results of PCR tests to identify of mecA gene of *Staph .aureus.*

Lane	Material and conc	MIC	Presence of mecA gene
1	thymus oil 100%	0.096 µg/ml	Negative
2	thymus oil 100%	0.0 48 µg/ml	Negative
3	thymus oil micro emulsion 20%	0.156 µg/ml	Negative
4	thymus oil micro emulsion 20%	0.078 µg/ml	Negative
5	cumin oil 100%	1.562 µg/ml	Negative
6	cumin oil 100%	0.781 µg/ml	Negative
7	cumin oil micro emulsion 20%	10 µg/ml	Negative
8	cumin oil micro emulsion 20%	50 µg/ml	Negative
9	Mix thymus , cumin oil micro emulsion 20%	1.25 µg/ml	Positive
10	thymus oil 20%	0.312 µg/ml	Positive
11	cumin oil 20%	10 µg/ml	Negative
12	cumin oil 20%	5 µg/ml	Positive
13	cumin aqueous extract 20%	20 µg/ml	Positive

Table 5: Results of PCR tests to identify of co-coagulase gene of *Staph aureus*.

Lane	Material and conc	MIC	Presence of co- agulase gene
1	thymus oil 100%	0.0 48 µg/ml	Negative
2	thymus oil micro emulsion 20%	0.156 µg/ml	Negative
3	cumin oil 100%	1.562 µg/ml	Negative
4	cumin oil micro emulsion 20%	10 µg/ml	Negative
5	Mix thymus , cumin oil micro emulsion 20%	0.625 µg/ml	Negative
6	thymus oil 20% conc	0.312 µg/ml	Negative
7	cumin oil 20%	10 µg/ml	Negative
8	cumin aqueous extract 20%	20 µg/ml	Negative



(a) **(b)**

Fig.4: a. Results of PCR assays for identification of mecA gene of *Staph .aureus.*
Lane M: DNA Ladder, Lane -ve: control Negative, Lane +ve: control positive,Lane 1,2,3, 4,5, 6, 7,8,11: negative. Lane 9, 10,12,13,14: Positive.

b. Results of PCR assays for identification of co-coagulase gene of *Staph aureus*.
Lane M: DNA Ladder, Lane +ve: control positive,Lane -ve: control Negative. Lane 1,2,3,4,5,6,7,8: negative.

Transmission electron microscope (TEM)

After incubation with MICs of 20% thyme and mixed nanoemulsion, TEM showed that the bacteria shortened and cell wall membrane was destroyed and the nanoemulsion lead to nucleus disruption and leakage of cellular contents **Fig. (5 a, b and c).**

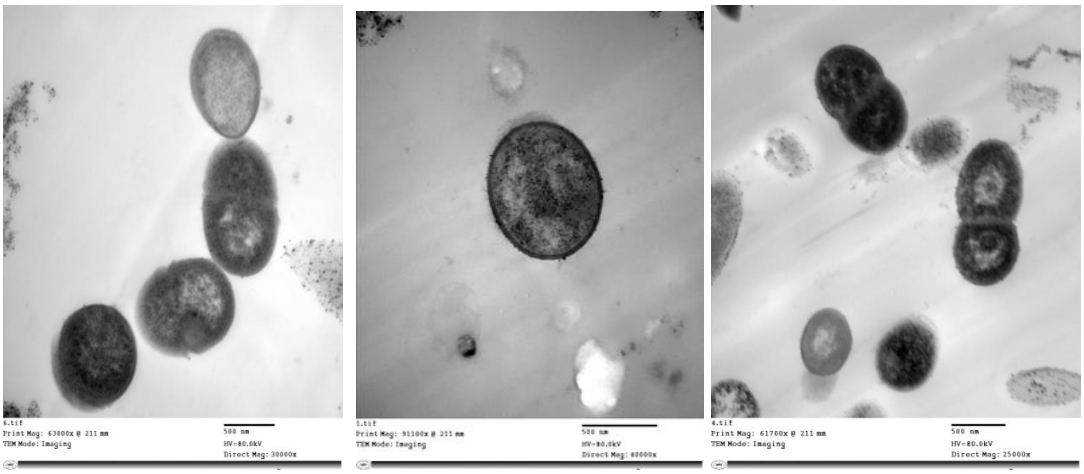


Fig 5. a: control staph. aureus under TEM.

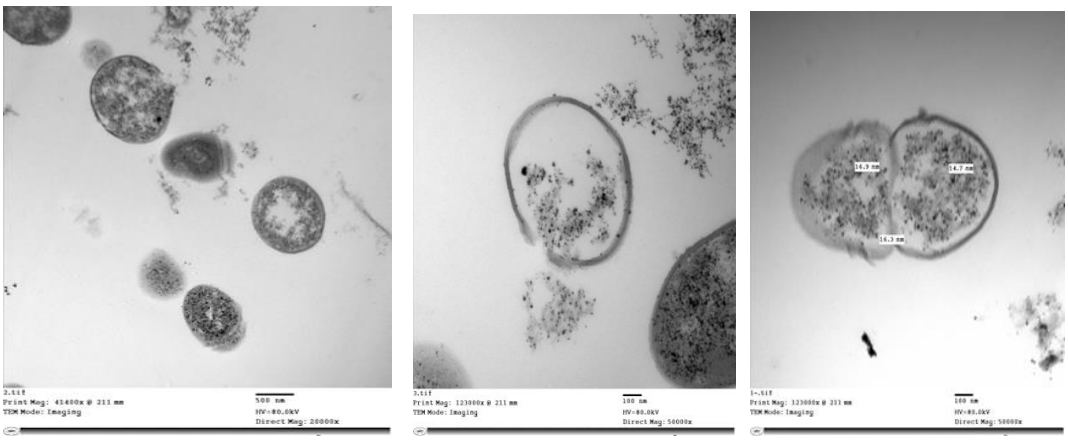


Fig. 5: b . *S.aureus* after treatment of Thyme oil nanoemulsion under HRTEM.

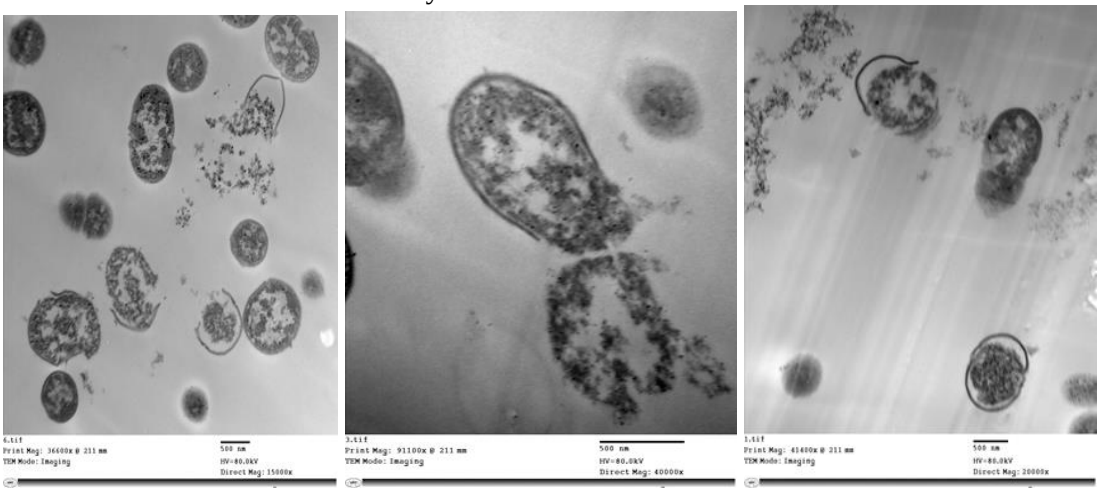


Fig. 5: c. *S.aureus* after treatment of mix cumin and thyme nano emulsion under HRTEM.

Discussion

When we started this study, the research plan was based on five materials, which are extracts of two oily plants, cumin, and thyme, with a concentration of 100%, and three nano-emulsions made from them, which are nano-cumin, nano-thyme, and a nano mixture of cumin and thyme with a concentration of 20% for each one. Our research is to find alternatives to antibiotics, as well as for food preservation, and to obtain the lowest concentration with the highest effectiveness to prevent or dicrease of side effects. Therefore, all tests for the characterization of nanomaterials have been done for only three materials and chemical analysis of cumin and thyme oil extracts. Where the idea of research depends on the comparison between the state of matter In its natural state and between its nano state in terms of its concentration and effectiveness on microbes. During the work, we saw that the concentrations should be equal between the two cases, and for this, we made other nanomaterials with the same concentration. After that, we have work aqueous extracts from the same plants and made nanomaterials from them with similar concentrations in the oily state, to be a comprehensive study on these plants to obtain the best result among them in terms of their effect on salmonella bacteria, Staphylococcus aureus, and their genes.

Staphylococcus aureus is a serious infection-causing bacteria that readily develop drug resistance. Even S. aureus that is drug-susceptible can live and remain on antibiotic therapy, necessitating protracted treatments and perhaps requiring surgical operations. These so-called persists have arrested-growth characteristics and can withstand large doses of antibiotics[5]. Plant extracts are found to be valuable antimicrobials that can be used as preservatives to food without any side effects . this study indicated that essential oil has better effect as an antibacterial than the watery extract this is because essential oils are volatile, natural, complicated chemicals with a sturdy odor that is generated by aromatic plants as secondary metabolites. They are lipid-soluble inorganic solvents having a density that is less than that of water. that are volatile, clear, and infrequently colored oily liquids., limpid, and rarely colored. All plant components, including seeds, fruits, roots, timber, bark, buds, flowers, leaves, stalks, and twigs. can produce these oils, which are then deposited in secretory cells, cavities, canals, epidemic cells, or glandular trichomes. Antimicrobial, antifungal, antiviral, insecticidal, and antioxidant effects have been discovered in them[6&7&8] and[26] find out which bacteria species the essential oil of cumin is effective against. included the highest antimicrobial activity of the EO observed against *Staphylococcus aureus*, with MIC values of 0.63 mg/mL

And[27] looked at the antibacterial properties of cumin essential oil against a variety of bacterial species, such as *E. coli*, *S. aureus*, and *S. faecalis*. The outcomes showed that the essential oils of cumin had inhibitory zones of 13, 10, and 10.33mm, respectively.

In our study, the chemical analysis of essential oil revealed that Thyme oil had nine chemical substances, 20% thyme nanoemulsion had seven chemical components, and cumin oil had nine chemical components whilst, 20% cumin nanoemulsion had eight chemical components, all these constituent was mentioned in result, Most of this component showed important broad-spectrum biological activities. A broad spectrum effect of these bio activities was mentioned by many researchers[28&29].

Thermodynamically settled thyme nanoemulsions were studied for antimicrobial activity versus Zygosaccharomyces bailii, an acid-resistant spoilage yeast (ZB). The oil stage proportion (ripening inhibitor kind and concentration) had a significant effect on the antibacterial action of the thyme oil nanoemulsions and acceded with previous research [30].Nanoemulsions enable the usage of essential oils in food products by rising their dispersibility in eating areas where pathogens sow and replicate, lowering their impact on product quality attributes, and improving their antimicrobial activity.

The result of this study showed that thyme oil nanoemulsion in a concentration of 20% has a MIC of 0.156 µg/ml which was higher than the MIC of thyme oil alone even in a concentration of 100%. Cumin oil nanoemulsion showed also higher MIC than Cumin oil. Mix thyme, and cumin oil nanoemulsion, also resulted in higher MIC in

comparison with other oils alone. These results indicate that the nanoemulsion of thyme and cumin essential oils has antimicrobial activity against *S.aureus*, this agreed with the result of[31].and showed the values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Thyme essential oil bacteriostatic and bactericidal effect against S. aureus[32] **and agreed with[33]** It was found through the minimum inhibitory concentration (MIC) significant effect of thyme essential oil nanoemulsion on Staphylococcus aureus (gram-positive bacteria)

The impact of the chemical in the nano form was discovered to be stronger in a lower concentration than that of the regular aqueous extract and essential oil. .

perception of how nanoemulsions influence the charge transport of EOs to the cell membrane and the mechanism of antimicrobial work will aid in the creation of more efficient distribution systems and focus on promoting the utilization of EOs in feed systems[15]. Dialysis tubing with two distinct pore volumes has been utilized, one without Nano emulsion droplet or micelle delivery enables antimicrobial compounds to be delivered only through the aqueous environment, whilst the other utilized both the watery phase and micelles[16].

When micelles were present in origanum oil nanoemulsions, the distribution of all antimicrobial agents was more efficient[34].These emulsions offer additional benefits due to their nanometer size range and thermodynamic features, which make them suitable for use as efficient drug delivery systems. The ongoing research on essential oil micro- and nanoemulsions' antibacterial properties and their use as medication delivery systems. On the quality of white soft cheese, cumin essential oil (CEO) nanoemulsion usage as a brined solution was observed. Significant antibacterial action was shown by CEO nanoemulsion against a variety of pathogens, including Staphylococcus aureus[14].

In this study the application of TEM showed that the impact of nanoemulsion caused cell membrane harm and destruction, these results are similar to those of[23,35] who detected also damage to bacterial cell as a result of thyme nanoemulsion effect on bacteria.

To better understand the mechanism of antimicrobial activity of thyme (Thymus vulgaris), researchers measured changes in internal pH (int) and membrane potency in Staphylococcus aureus cells next exposure to the plant extract. The results showed that plant extract had a significant effect on the cell membrane gram-positive bacteria, as evidenced by a relief in pain and cell membrane hyperpolarization[36].

Conclusions

the plant extracts and nanoemulsions used in this study have high effect at the reduction of bacterial growth . so it can be used effectively as a food preservative for the prevention of foodborne diseases without any health problems that associated with chemical preservatives .

Declarations

Ethics approval and consent to participate

According to standards for animal welfare, this work was authorised by the Institutional Animals Care and Use Committee, Research Ethics Board, Faculty of Veterinary Medicine, Benha University (No. BUFVTM 34-10-22).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Author Contributions

The design and conceptualization of the study were contributions from all authors.SA, A A, and DE prepared the materials, collected the data, and carried out the analysis.SA, WA, and FE wrote the first draught of the text, and they all provided feedback on earlier draughts. The final manuscript was reviewed and approved by all writers.

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مستحلب نانوي الزعتر والكمون كعامل واعد مضاد للميكروبات ضد المكورات العنقودية الذهبية المقاومة للأدوية المتعددة

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هدفت هذه الدراسة إلى مقارنة مستخلصات الزعتر والكمون ومستحلبات النانو كمضادات للبكتيريا على بكتريا المكورة العنقودية البرتقالية. تشمل الطرق تحضير المستخلص المائي والزيت ومستحلب النانو من الزعتر والكمون ، وتحديد (MIC باستخدام طريقة) (resazurin microdilution)، واكتشاف جينات mecA و (coagulase) بواسطة تفاعل البلمرة المتسلسل قبل وبعد العلاج باستخدام مستحلبات النانو ، وتحديد البكتيريا بعد العلاج باستخدام الميكرسكوب الالكتروني . أظهرت النتائج أن قيم (MIC) لزيت الزعتر ١٠٠٪ ، مستحلب نانو زيت الزعتر ٢٠٪ ، زيت الكمون ١٠٠٪ ، زيت الكمون مستحلب نانو ٢٠٪ ، مزيج الزيت ٢٠٪ ، زيت الزعتر ٢٠٪ ، زيت الكمون ٢٠٪ (٠,٠٤٨ ، ٠,١٥٦ ، زيت الكمون . ١,٥٦٢، ١٠، ٠,٦٢٥، ٠,٣١٢، ١٠) mg / m على التوالي ، غابت جينات mecA و (coagulase) بعد العلاج في المختبر وأظهرالميكرسكوب الالكتروني البكتيريا مع تلف كبير في غشاء الخلية. أظهرت النتائج أن مستحلب النانو له تأثير أعلى على بكتريا المكورات العنقودية مقارنة بالزيت العطري وحده.