Antibacterial Activity of Eucalyptus Essential Oil loaded on Silica Dioxide Nanoparticles (SiNPs) Against Some Pathogenic Bacteria

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ABSTRACT

Essential oils (EOs) have been used in the food industry, pharmaceuticals, and cosmetics since they are pure and have special aromatic qualities and are bactericidal, fungicidal, insecticidal, and have many other therapeutic advantages. Numerous studies have established nanoparticles (NPs) ability with wide spectrums, including antioxidants, anticancer and antimicrobial properties, for both EOs with metal/metal oxides NPs. The study aims to evaluate a dual combination therapy's antibacterial efficacy using Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs) against certain pathogenic bacteria. The antibacterial activity of the eucalyptus essential oil, SiNPs only, and its encapsulated form SiNPs against gram-positive (MRSA and S.aureus) and gram-negative bacteria (P.aeruginosa and E.coli) were investigated. The agar well diffusion method determined the antimicrobial activity of the eucalyptus EOs with and without SiNPs.Eucalyptus essential oil encapsulated with silica dioxide nanoparticles showed high antibacterial activity against MRSA, S. aureus, P. aeruginosa, and E. coli. In the present study, SiNPs only exhibited a significant increase in diameters of inhibition zones against S. aureus and MRSA compared to the eucalyptus essential oil. aeruginosa compared to the eucalyptus essential oil only. However, Eucalyptus essential oil loaded on silica dioxide nanoparticles revealed a significant increase of the diameters of inhibition zones against both gram-positive bacteria and gram-negative as compared to eucalyptus essential oil only and SiNPs only. The present study suggested that the potent efficacy of Eucalyptus oil against both gram-positive bacteria (MRSA and S. aureus) and gram-negative bacteria (P. aeruginosa and E. coli) could be improved by silica dioxide nanoparticles (SiNPs).

Key Words: *Essential oil, Silica dioxide nanoparticles, Antibacterial, Gram-negative bacteria, Gram-positive bacteria, Eucalyptus oil*

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INTRODUCTION

Antimicrobial resistance has been a major threat to the world's public health services in the last two decades. The misuse and exploitation of antimicrobials in veterinary and human medicine have intensified the increasing global phenomenon of antimicrobial resistance since the antibiotic age, with the discovery of the first antibiotics bringing consistent health benefits to human medicine [1-3].

The most opportunistic and life-threatening bacteria causing significant infections, in particular in patients with an immune risk are *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) [4]. Post-operative wound inflammation, toxic shock syndrome, and food poisoning are usually caused by *S. aureus*. In humans, *E. coli* causes lower urinary tract inflammation or septicemia [5]. Many researchers reported an increase in resistance to antibiotics in *S. aureus* and *E. coli* [6-8]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most severe resistant pathogen among gram-positive bacteria for nosocomial infections. Most of the antibiotics that have been used in the past have been less successful against these bacteria. Therefore, Alternate antimicrobial agents are also urgently needed for the treatment of resistant pathogenic microorganisms [9].

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Natural substances derived from plants, such as essential oils (EOs), are used in the food industry to protect fragile products against oxidative processes in a variety of applications, such as the release of perfumes or active substances in cosmetics [10]. Among the natural bioactive agents with promising antimicrobial activity, EOs have recently attracted interest [11]. EOs are a mixture of volatile constituents generated as secondary metabolites by aromatic plants as a defense measure against pests, microorganisms, or weather adversity [12]. However, during application or storage EOs activity i.e., liability and volatility, decreases [13].

Eucalyptus is one of the most important and widely distributed plants all over the world [14]. Eucalyptus trees are popular because of their healing and medicinal properties. However, the most common and represented species in international pharmacopeia is the *Eucalyptus globulus (E. globulus)*, the major provider of Eos [15]. Eucalyptus EOs have antibacterial, antifungal, analgesic, and anti-inflammatory effects and have been commonly used in pharmaceutical, nutritional and cosmetic products [16-18].

Nanotechnology has proven to be an effective method to solve numerous medicinal and technical problems using its predetermined structures [15]. One such technique that could theoretically counteract the setbacks mentioned above is to use NPs to supply antimicrobials. The benefits of nanomaterials in this application are numerous. The pathogens' mode of uptake can be tailored by using NPs as carriers and thereby circumvent problems associated with the mechanism of antimicrobial resistance such as hyperactive efflux pumps [19, 20]. Silica dioxide nanoparticles (SiNPs) are a special class of inorganic nanoparticles with broad different functional characteristics that are beneficial [21]. Due to their simple preparation and wide use in various industrial applications, such as pharmacy and electronic insulators, SiNPs play a significant role in scientific research [22]. The flexibility of SiNPs is particularly valuable for antimicrobial therapeutics, considering the growing challenge of antimicrobial resistance. The window for the development of antimicrobial resistance is very short, in addition to the antimicrobial activity caused by the cargo itself, as these nanoparticles can attack pathogens in many modes, including physical damage to cell membranes, the formation of reactive oxygen specious, and endo-lysosomal burden [1]. Nevertheless, studies related to metal oxide nanoparticles against gram-positive and gram-negative infectious pathogens are too limited. Thus, the current research is designed to evaluate a dual combination therapy's antibacterial efficacy using Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs) against certain pathogenic bacteria.

MATERIALS AND METHODS

Materials

The following chemicals and solvents were used: Silicon Dioxide Nanoparticles (SiO_2NPs) were purchased from NanoTech Egypt for Photo-Electronics, 6th of October City, Giza, as a white powder, of size ≈ 50nm ± 10. The pure essential oil from *Eucalyptus globulus* (eucalyptus*)* and Triton X-100 were purchased from Sigma Aldrich® USA. Mueller-Hinton agar and Mueller-Hinton Broth were purchased from Fluka®, India.

Synthesis of silica nanoparticles (SiNPs) and oil encapsulation

In the case of oil encapsulation, a capped tube was used for the usage of 10mg of powdered silica nanoparticles, and the porosity was increased by 100µl of the Triton X-100. 1000µl Eucalyptus globulus oil has been applied to the above solution. A magnetic stirrer has been actively stirring this solution for 4 hours. The suspension was then subjected to a centrifuge for 10 minutes at 10,000 rpm, which produced the pellet which was stored overnight at room temperature.

Microscopic examination of silica dioxide nanoparticles and the eucalyptus essential oil encapsulated silica nanoparticles

Transmission Electron Microscopes (TEM JEOL-JEM-1010 at 80 kV) were used to evaluate the distribution of size, shape, and aggregation state SiNPs. Nanoparticles were ground and diluted in phosphate buffer saline, forming a suspension that was deposited on carbon-coated copper grids. The grids were allowed to dry at ambient temperature before analysis by electron microscope [23].

Bacterial strains

Gram-positive bacteria such as *Staphylococcus aureus (S. aureus)* (ATCC 29213), Gram-negative bacteria such as *Escherichia coli (E. coli)* (ATCC 25921), *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 27853), and Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 52923) were used to evaluate the antibacterial activity. All bacterial strains were provided from the microbiology laboratory, Ain shams University Hospital, Cairo, Egypt.

Preculture preparation of bacteria

In Mueller-Hinton broth, strains previously described and preserved have been taken and inoculated. The suspension was taken and shook well with the vortex at 37°C for 24 h. For standardization, then diluted. The inoculum was placed at 0.5 McFarland or 0.08 optical density. At a concentration equal to 0.5 McFarland 0.08 to 0.13 at 625 nm wavelengths, which correlates to 10^8 CFU/mL, bacterial suspensions were prepared [24].

Agar well diffusion method

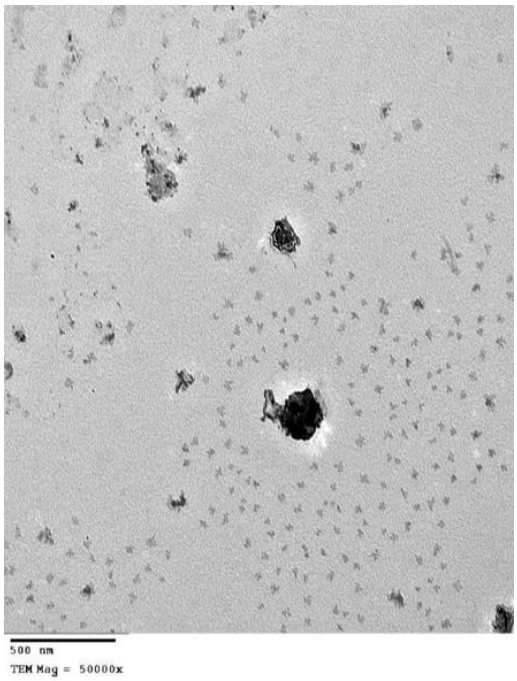
The antimicrobial activity of eucalyptus EOs with and without SiNPs was determined by the agar well diffusion method which is based on the spread of antimicrobial compounds in a solid medium [25]. The Mueller–Hinton agar was poured into sterile Petri dishes (85 mm×15 mm). About 0.1 ml of an inoculum suspension (tested bacterium, 10^8 CFU/ml) was poured and spread using a cotton swab. After inoculum absorption by agar, wells were made using sterile cork poorer (diameter 5 mm) and were filled with 50 µl of the eucalyptus Eos. The Petri dishes were placed at room temperature at 37°C for 30 minutes, 24 hours before the incubation. The effect of eucalyptus EOs alone was reflected by a transparent circular zone, which correlates to a lack of growth. The inhibition zone diameter was estimated in mm, and the average value was determined. The broader the area's diameter the more sensitive the strain is [26]. The same was done by using SiNPs and eucalyptus EOs encapsulated a SiNPs. All experiments were carried out in triplicate. Müller–Hinton agar of cultured induced for 24 hours at 37 ˚C without adding EOs, SiNPs, and eucalyptus oil encapsulated SiNPs with the bacterial strain used as control.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Science package (Version 26, SPSS INC., Armonk, NY: IBM Corp). The one-way variance analysis (ANOVA) followed by the post-hock least significant (LSD) test was used to compare variables. All Values were expressed as mean ± standard error of the mean (SEM). $P < 0.05$ was considered significant. GraphPad Prism 9.00 Applications for GraphPad, La Jolla California USA, www.graphpad.com" have created the graphs.

RESULTS AND DISCUSSION

On examination of SiNPs by TEM, they appeared as tiny electron-dense particles of a relatively similar size range. They appeared nearly rounded, solid, and non-porous. While eucalyptus essential oil encapsulated in the SiNPs tended to coalesce together, forming variable-sized aggregates. The average size of encapsulated SiNP was 20 to 70 nm **(Figure 1)**.



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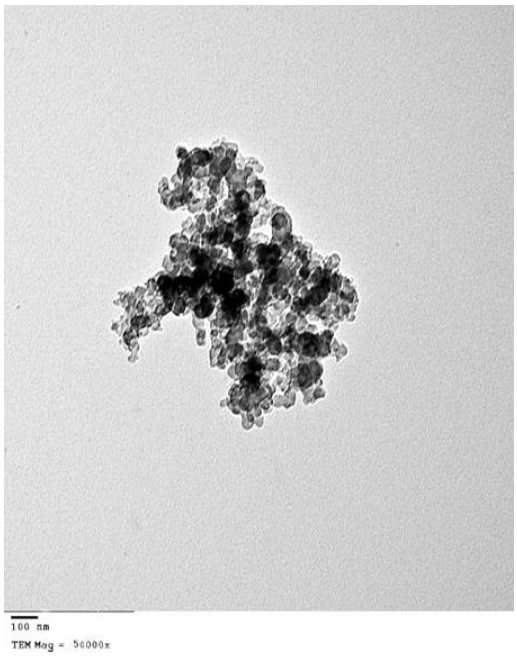


b)

Figure 1. a) A transmission electron micrograph of SiO_2 NPs showing non-porous rounded, electron-dense particles. b) Eucalyptus essential oil encapsulated SiO_2 NPs. Electron transmission micrographs display nanoparticles aggregations of varying sizes (TEM x 50000).

Essential oils currently displayed variable activities towards almost the tested bacterial strains**.** Antibacterial activity was determined by measuring the diameter of the inhibition area. The antibacterial activity of Eucalyptus essential oil alone, Silica dioxide nanoparticles alone, and

Eucalyptus essential oil loaded on silica dioxide nanoparticles were summarized in **(Table 1 and Figures 2 and 3)**.

Table 1. The antimicrobial activity of the Eucalyptus essential oil, Silica dioxide nanoparticles (SiNPs), and Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)against different tested bacteria.

		N	Mean± SEM
Staphylococcus aureus (S. aureus)	Eucalyptus essential oil	3	10.83±.44
	Silica dioxide nanoparticles (SiNPs)	3	17.66±.33 ***
	Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)	3	24.16±.60****, ###
Escherichia coli (E. coli)	Eucalyptus essential oil	3	6.83±.16
	Silica dioxide nanoparticles (SiNPs)	3	7.83±.16 (ns)
	Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)	3	11.66±.88**, ##
Pseudomonas aeruginosa (P. aeruginosa)	Eucalyptus essential oil	3	12.33±.33
	Silica dioxide nanoparticles (SiNPs)	3	14.00±.57 (ns)
	Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)	3	17.33±.33 ***, ##
Methicillin-resistant *Staphylococcus aureus* (MRSA)	Eucalyptus essential oil	3	11.33±.66
	Silica dioxide nanoparticles (SiNPs)	3	14.33±.33*
	Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)	3	18.66±.33***, ##

Data were expressed as mean± standard error of the mean (SEM), N= number of the trials, One-way ANOVA followed by Least significant difference (LSD) comparison tests. ns=non-significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, respectively compared to Eucalyptus essential oil alone. ## P < 0.01 and ### P < 0.001 respectively compared to Silica dioxide nanoparticles (SiNPs) alone.

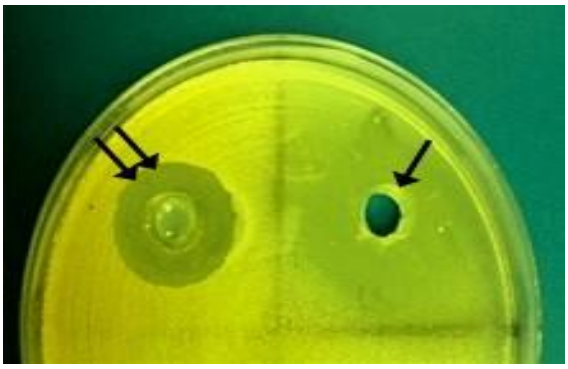
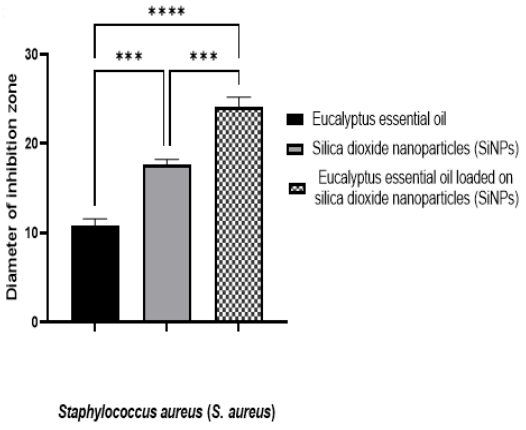


Figure 2. Inhibition zone of eucalyptus essential oil alone (↑) and eucalyptus essential oil encapsulated Silica dioxide nanoparticles (SiNPs) (↑↑) against *S. aureus.*



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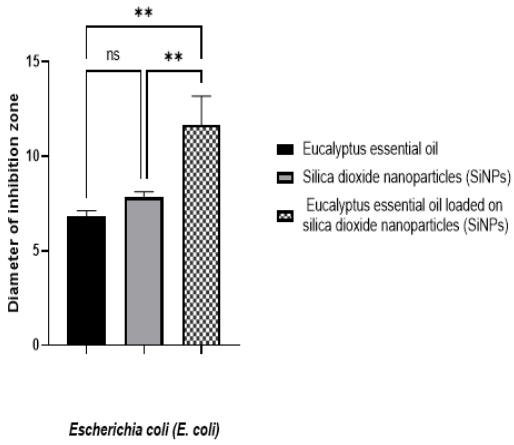

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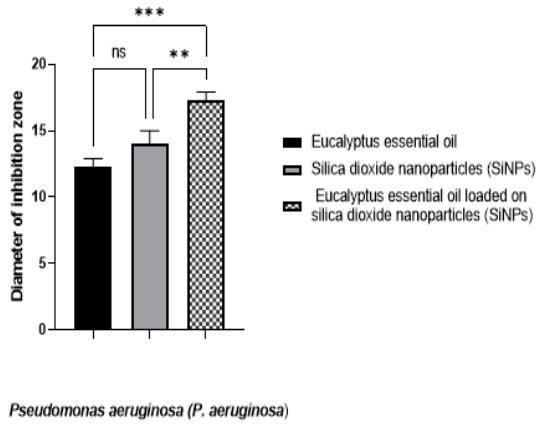

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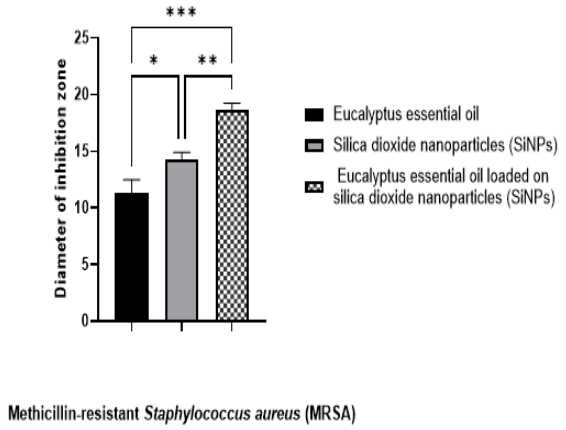

d)

Figure 3. The antimicrobial activity of the Eucalyptus essential oil, Silica dioxide nanoparticles (SiNPs), and Eucalyptus essential oil loaded on silica dioxide nanoparticles (SiNPs)against different tested bacteria. A: *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa),* and Methicillin-resistant *Staphylococcus aureus* (MRSA). ns=non-significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

In the present study, the results revealed that Eucalyptus EO had antibacterial activity gram-positive bacteria (MRSA and *S. aureus*) with diameters of inhibition zones of 11.33±0.66 mm and 10.83±0.44 mm; respectively. Moreover, it showed antibacterial activity against *P. aeruginosa* and *E. coli* with the diameter inhibition zone 12.33±0.33 mm,6.83±0.16 mm, respectively. The highest activity was found against MRSA, *P. aeruginosa,* and *S. aureus,* respectively whilst the least antibacterial activity appeared with *E. coli.*

When regarding silica dioxide nanoparticles alone, the antibacterial activity appeared high against *S. aureus*, MRSA, and *P. aeruginosa* with diameters of inhibition zones of 17.66±0.33 mm, 14.33 ± 0.33 mm, and 14.00±0.57 mm; respectively. At the same time, the least antibacterial activity appeared against *E. coli* with the diameter of inhibition zones of 7.83±0.16 mm.

Interestingly, Eucalyptus essential oil encapsulated with silica dioxide nanoparticles showed high antibacterial activity against both MRSA, *S. aureus*, *P. aeruginosa*, and *E. coli.* The highest appearing against *S. aureus*, MRSA, and *P. aeruginosa* with diameters of inhibition zones of 24.16±0.60 mm, 18.66 ± 0.33 mm, and 17.33±0.33 mm; respectively. In comparison, the least antibacterial activity appeared against *E. coli* with the diameter of inhibition zones of 11.66±0.88 mm [27, 28].

In the present study, SiNPs only exhibited significant increase diameters of inhibition zones against *S. aureus* (P < 0.05) and MRSA (P < 0.001) in contrast to the eucalyptus essential oil only. There was a non-significant (P > 0.05) difference of diameters of inhibition zones against E. coli and P. aeruginosa compared to the eucalyptus essential oil only. However, Eucalyptus essential oil loaded on silica dioxide nanoparticles revealed significant increase of the diameters of inhibition zones against both *S. aureus* (P < 0.0001, P < 0.001; respectively) and MRSA (P < 0.001, P < 0.01; respectively) and *E. coli* (P < 0.01, P < 0.01; respectively) and *P. aeruginosa*) (P < 0.001, P < 0.01; respectively) as compared to eucalyptus essential oil only and SiNPs only.

In general, our results highlighted that the eucalyptus essential oil-based nanoparticles act as a potent antibacterial against both gram-positive and negative bacteria when compared with SiNPs only and eucalyptus essential oil alone.

Owing to the widespread use of antibiotics to combat infectious diseases without sufficient clinical guidance, bacterial resistance has become a tremendous concern. Alternative antibacterial agents have drawn significant concern about solving this problem. Old and new antimicrobial compounds, such as metallic NPs, appeared to be the most powerful agents for fighting pathogenic bacteria over the past several decades. Because of its large surface area to volume ratios, metallic NPs have strong, targeted, and sustained antimicrobial activity with bacteria at lower doses [29, 30].

Many EOs, like eucalyptus, were used worldwide for traditional medicine and their medicinal properties were studied. Eucalyptus EO has antibacterial, antifungal, analgesic, and anti-inflammatory properties and was commonly included in herbal, dietary, and cosmetic products [31]. The results revealed that eucalyptus EO had antibacterial activity against gram-positive and negative bacteria in the present study. The high activity was found against MRSA, *P. aeruginosa*, and *S. aureus;* respectively, and the least antibacterial activity appeared with *E. coli.* These findings are almost comparable to those seen in other experiments on the antimicrobial function of *E. globulus* leaf essential oil as well as those of related organisms [15, 32], and validate their conventional uses [33]. EOs show a broad inhibition effect towards different pathogenic bacteria by quickly splitting the lipids of the bacterial cell membrane and destroying their cell wall structure [34]. The interaction of lipid constituents of EOs induces loss of cellular material and eventually contributes to bacterial cells' death [35]. In the present study, we reported that eucalyptus Eo showed a high diameter of inhibition zone for *P. aeruginosa* than *S. aureus.* Some authors have reported that as compared to gram-positive, gram-negative microorganisms are significantly more sensitive to Eos [36]. In different ways, gram-positive and gram-negative microorganisms vary in their cell wall composition, especially concerning the lipoprotein and

lipopolysaccharide existence in gram-negative bacteria, representing barriers for hydrophobic compounds [23].

TEM has studied the surface morphology of the SiNPs encapsulated. The SiNPs, with a scale varying from 20 to 70 nm, were mainly spherical and rounded in shape. The study confirms the result of a previous Dohare *et al.* study, which reported that the SiNPs encapsulated with eucalyptus essential oil was 1000 nm in scale, spherical form, and well distributed in water [37]. Researchers also stated that the eucalyptus essential oil encapsulated in SiNPs had a rounded form and a mean diameter of 0.70 µm [38]. The structure, size, and regulated disparity of NPs play a significant role in evaluating the properties assigned to their applications in the field of biomedicine [25]. The antibacterial efficacy of NPs differed as their sizes reduced, researchers observed [26].

In the present study, SiNPs only exhibited antibacterial activity against gram-positive and negative bacteria in contrast to the eucalyptus essential oil. The antimicrobial properties of metal oxide NPs have been studied by only a few studies [39, 40]. Researchers stated that metal oxide-based NPs destroy the cell membrane by metallic ions binding and releasing them into the bacterial cell wall. Via multiple modes of action, such as electrostatic attraction and hydrophobic interactions, the bacterial cell wall could be destroyed by NPs [41]. Even so, there are different kinds of NPs with various means for combating bacteria by creating pores on the surface of the bacterial cell membrane, which consequently results in to release of free radicals, reactive oxygen, oxidative stress, and changes in the levels of gene expression [31]. Also, researchers reported that SiO_2 NPs nanocomposites exhibited superior antimicrobial activity against both susceptible and tetracycline antibiotics -resistant E. coli strains [42].

There are also other studies on different NPs' antibacterial effects, but some reports have been conflicting [43-45]. These studies stated that the mechanisms of toxicity of NPs are massively complex and rely on different factors like structure, surface alteration, intrinsic properties, and species of bacteria. The exact reasons for the toxicity of NP of different bacteria are not well known. By electrostatic activity, NPs will bind to the bacteria's membrane and interrupt the bacterial membrane's integrity [46]. Usually, nanotoxicity caused oxidative stress induction after administration of NPs through the formation of free radicals [47].

Essential oils have demonstrated strong antimicrobial properties. Even so, due to poor water solubility and its high susceptibility to oxygen, humidity, heat, and light, its use is very limited. To enhance their stability, water solubility and prevent EOs from oxidation, multiple alteration developments have emerged as solutions to these current problems. Models used to encapsulate natural bioactive molecules, improving antimicrobial activity, including the encapsulation of EOs into nano-based structures, such as nanoemulsions and microemulsions, solid-lipid NPs, and liposomes. At present, the use of nanoencapsulation technologies has grown rapidly due to its exciting parameters, such as size, zeta potential, and polydispersity index [48]. In the present study, eucalyptus EOs loaded on silica dioxide nanoparticles revealed a significant increase in the diameter of inhibition zone against both gram-positive and negative bacteria in contrast to eucalyptus essential oil only and SiNPs only. These findings are almost identical to those shown by other research on the antimicrobial behavior of eucalyptus essential oils containing nanoparticles of silica dioxide [49-51]. Researchers measured E. globulus oil's impact on *E. Coli* biofilm and it has been shown to suppress the biofilm by 62 percent. Evaluated even the influence of SiNP-containing oil on the biofilm system, *E. globulus* oil was loaded into the SiNPs. The use of oil-loaded SiNPs was made against the *E. coli* biofilm method and biofilm quantification assay showed a decrease of about 81 percent in biofilm [52, 53]. The inhibition was further confirmed by a light microscopic examination of the 0.5 percent crystal violet biofilm after staining. They concluded that the use of Eucalyptus globulus oil encapsulated within the nanoparticle is valuable for its future use in the prevention and management of biofilm-related microbial infections and diseases [54].

CONCLUSION

Rising antimicrobial resistance and the lack of a range of therapeutic approaches also contributed to the rising importance of creating nanotechnology-based therapeutic approaches for bacterial infections. The interest in designing biomedical devices that incorporate therapeutic and antibacterial capacity is growing. In this area, silica-based nanostructured materials seem especially promising as they combine mechanical stability and biocompatibility with very flexible chemistry that enables measurements, morphology, and surface properties to be controlled. Therefore, by encapsulation processes, antibacterial agents may be produced that locally kill bacteria without being harmful to surrounding cells and tissues. Overall, the research suggested that the potent efficacy of Eucalyptus oil against MRSA, *S. aureus*, *P. aeruginosa*, and *E. coli* could be improved by silica dioxide nanoparticles (SiNPs).

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Conflict of interest: None

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Ethics statement: The experiment was conducted in the research center institute (MASRI), faculty of medicine, Ain shams university, Research Ethics Committe (FMASU REC) with federal wide Assurance No. FWA 00017585.

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