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In-vitro Antimicrobial Activity of Garlic Oil-Loaded Solid Lipid Nanoparticles Against Selected Pathogens

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ABSTRACT

For thousands of years, people have utilized garlic (*Allium sativum* L) as a medicinal ingredient. The low solubility of garlic essential oil (GO) has restricted the usage of garlic despite its several biologically active components and its ability to function as a fungicide and antibacterial. Solid lipid nanoparticles (SLNs) are colloidal drug carriers that could be used to formulate GO in order to overcome its poor solubility and potentially be a good delivery system for GO. This study was to determine the efficacy of GO-SLNs against three specific pathogens: *Candida albicans*, *Escherichia coli*, and *Methicillin-Resistant Staphylococcus aureus* (*MRSA*). High-shear homogenization and ultra-sonication methods were used to prepare GO-SLNs. The manufactured formulation's encapsulation efficiency, loading capacity, and physicochemical characteristics were evaluated. Scanning electron microscopy (SEM) was used to analyze the morphology of GO-SLNs (SEM). Using GC/MS analysis, the chemical composition of the tested normal oil and GO-SLNs was assessed. In vitro studies were conducted to compare the antibacterial activity of GO-SLNs and regular GO. The particle size of GO-SLNs was 110.5±3 nm with PDI of 0.320±0.04 and zeta the potential was -36.6±0.5 mV. The majority of GO-SLNs had a less structured crystal structure, which contributed to improving the drug loading capacity. GO-SLNs had an encapsulation efficiency of 88±0.70% and a loading capacity of 0.56±0.0. According to the results, the GO-SLNs inhibited the growth of MRSA, E. coli, and Candida albicans by 74%, 54%, and 85%, respectively. In addition, GO-SLNs had greater antibacterial activity than regular GO. According to the study's findings, changes need to be made to the loading capacity, encapsulation efficiency, and physicochemical characteristics. Our findings demonstrated that the SLNs were effective GO carriers for the control of bacterial and fungal diseases and that more research should be done on them.

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INTRODUCTION

Based on the unique organo-sulfur compounds, the garlic (Allium sativum L) plant is widely used as a food flavoring and is believed to have a good effect on human well-being and health [1]. The main components of garlic oil are diallyl disulfide, trisulfide, allyl propyl disulfide, disulfide, and most likely diallyl polysulfide [2]. Particularly, it has been discovered that the essential oils extracted from garlic exhibit favorable anti-carcinogenic, anti-diabetic, and anti-microbial activity. This is noteworthy for various pharmacologic and

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medicinal applications [3]. Moreover, they have the potential to lower blood pressure, cholesterol, and plasma aggregation [4].

Natural product-based medicines have attracted a lot of interest lately due to their potency against microorganisms and lack of medication resistance [5-8]. The antimicrobial action of garlic against many bacteria, viruses, and fungi is well documented [9-12]. Garlic has many sulfur compounds, which give it antibacterial properties. It is suggested that allicin, the main ingredient, possesses several ways of acting as an antibacterial. Antimicrobial drugs can inhibit or eliminate microorganism resistance in a variety of ways [13]. The main ingredient in garlic, allicin, has been shown to have antimicrobial activity through the following mechanisms: 1) membrane permeability and cell-structuring ability, [13,14,2] the capacity to modify the way that microbes express their genes, and 3) the reactivity with enzymes that contain thiols, which causes oxidative stress. [15-16].

Garlic oil's high volatility, potent smell, insolubility in water, and low physicochemical stability limit its application [17]. Based on the previously described factors, garlic oil's bioavailability in terms of systemic circulation decreases after oral intake. As a result, despite the many benefits that garlic oil offers, its use in the food, cosmetic, and pharmaceutical industries is severely restricted. Because of this, it has been suggested that to improve their industrial applications, nano-scale garlic oil microemulsions be used, lowering the dosage levels required [18]. This can be accomplished by increasing the oral bioavailability and dissolvability of water-soluble drugs like garlic oil by using lipid-based formulations. Because of its capacity to control, characterize, or create materials or devices with dimensions ranging from 1 to 100 nm, nanotechnology is used in this area in a very important way [19]. Nano-emulsions are usually smaller than 500 nm and have better absorption qualities because of the mucosa [20].

First-generation lipid nanoparticles made from a solid matrix, solid lipid nanoparticles (SLNs) can be identified by the presence of one or more solid lipids (saturated fatty acids). [21]. The shortcomings of previous lipid-based nanocarriers were thus solved with the development of SLNs. To achieve this, a solid lipid was added to the emulsion in place of the liquid lipid (oil), giving it an ordered crystalline structure and enabling the bioactive ingredients to be contained inside the lipid matrix.

One approach that shows promise for expanding the use of essential oils as antimicrobials is nanoencapsulation in solid lipid nanoparticles (SLNs) [22]. SLNs have been presented as a potential drug-delivery strategy for active components in cosmetics and pharmaceuticals because of their advantages over traditional formulations [23,24]. SLNs have special qualities like high drug loading, large surface area, and small size. They range in size from 50 to 1000 nm. In addition to protecting essential oils from environmental elements such oxygen, light, moisture, and acidity, SLNs can increase the stability and solubility of essential oils in water [25], increase the bioavailability of entrapped bioactive substances, and facilitate the controlled release of essential oils [26].

Extensive study on nano-scale garlic oil emulsification and its associated characteristics is lacking. Given that garlic oil functions as an antibiotic and an antifungal in addition to containing a variety of physiologically active chemicals. Garlic essential oil-containing SLNs (GO-SLNs) were to be obtained and characterized in this investigation. The in vitro antimicrobial efficacy of generated GO-SLNs was evaluated against selected representatives of Gram-positive (*Methicillin-Resistant Staphylococcus aureus* (*MRSA*),) and Gram-negative (*Escherichia coli*) and the fungus (*Candida albicans*) compared the antimicrobial effectiveness of regular GO antimicrobial activity.

MATERIALS AND METHODS

Materials

Garlic essential oil was obtained from Sigma-Aldrich. Glycerol monostearate was bought from Tianjin Chemical Reagent Stearic acid, Soybean lecithin, and tween-80 were supplied from Sigma-Aldrich, and dichloromethane was purchased from MERCK. D-95 distilled monoglyceride was from Guangzhou Jialishi Food Technology. Compritol 888 ATO was a bought from Cedex. The supplier of the Poloxamer 188 was BASF (Ludwigshafen Rhine, Germany). Shanghai Taiwei Pharmaceutical provided the lecithin.

Preparation of the GO-SLNs

SLN formulations were made using ultrasonic and high-shear homogenization techniques. After

heating Glyceryl mono stearate and Compritol 888 ATO to 70 °C, various volumes of GO essential oil were added to the lipid phase after the melting process to keep the essential oils from evaporating. The aqueous phase was created by dissolving 2.5% of either Poloxamer 188 or Tween 80 in 20 ml of double-distilled water, and then heating the mixture to the lipid phase's melting point. A Diax 900 homogenizer was used to combine hot aqueous and molten lipid phases and homogenize them for five minutes. The temperature was maintained at 5°C above the lipid's melting point. Using a probe sonicator, the resulting emulsion was ultrasonically treated. Six cycles of 30 seconds of sonication were carried out, with intervals of 15 seconds in between. After bringing the samples down to room temperature, SLNs were produced [27].

Characterization of GO-SLN

Particle size and zeta potential

The SLNs formulations were assessed for particle size, polydispersity index, and zeta potential using the dynamic light scattering method (ZetaSizer Nano-ZS) [28].

Determination of Encapsulation and Loading Efficiency

The encapsulation efficiency (EE) can be expressed as the percent of the total amount of GO obtained in the formulation at the end of the process. The mass of entrapped GO divided by the entire mass of lipid (stearic acid) is the loading capacity (LC). The EE and LC were determined as described earlier [29,30]. Ten ml of methanol was utilized to dissolve 10 mg of GO-SLN formulations, which were carefully weighed. After that, the samples were centrifuged for 30 minutes at 9,000 rpm. Utilizing a UV–Vis spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd.), the quantity of GO in the supernatant was measured at 274 nm. A calibration curve was created using a range of concentrations of pure garlic oil to determine the percentage of GO. Three duplicates of each concentration's measurements were made.

The encapsulation and loading efficiency determined as follows:

$$\%EE = (A-B)/A \times 100$$

$$\%LC = (A-B)/C \times 100$$

where;

A: The total amount of GO (% conc.) added to the formulation.

B: The amount of GO measured in the supernatant.

C: The total weight of lipid (stearic acid, 1% w/w) in the formulation.

Morphology Study

Scanning electron microscopy (SEM) has studied the nanoparticles' morphology. After being sputter-coated with a thin layer of Au-Pd, the nanoparticles were mounted on aluminum stubs and examined with an SEM (SEM XL30, Philips, Netherlands) [31].

Differential scanning calorimetry (DSC)

DSC scans of GO and GO-SLNs were carried out in a Mettler DSC 821e (Mettler Toledo, Germany). Five mg of the samples were placed into aluminum oxide pans, sealed, and subjected to analysis. As a guide, an empty aluminum pan was used. DSC was done at a 25 to 250 °C temperature range at the rate of 5 °C/min under N2 flow and the melting point of SLN dispersions was compared to the bulk lipid [32].

In Vitro Release Kinetics of GO in SLNs

The in vitro release study was carried out within 24 hours after preparing GO-SLN. Using the dialysis sack method and DO405 dialysis tubing 23–15 mm (Sigma, Germany), a release study was carried out. The dialysis membrane was filled with 5 ml of each formulation and submerged in 50 mi of phosphate buffer pH 7.4 with 0.1% Tween 80. The dialysis bag's medium was heated to 37° C and agitated at 100 rpm. At predetermined time intervals. Two ml samples were taken out (and replaced with an equal volume of fresh medium), and a UV spectrophotometer was used to measure the drug concentration at 424 nm. GO dispersion in the same concentration with GO-SLNs was investigated under the same release conditions to make sure that the sustained release profile is not the result of a membrane [31,33].

The Antimicrobial Study

Bacterial and fungal strains

The Lab of Microbiology, College of Science at Wasit University, supplied the strains used in the study, which included Gram-positive bacteria: *methicillin-resistant Staphylococcus aureus*

(*MRSA*) and Gram-negative bacteria: *Escherichia coli* and the *fungus Candida albicans.*

The antimicrobial activity of GO-SLNs

The practical part of this study was done in Wasit University's Faculty of Dentistry's microbiology lab. Mueller-Hinton agar was the culture medium that was produced for the study's bacterial cultures to grow in. Candida albicans was cultivated on Sabouraud Dextrose Agar (SDA). The well diffusion method was used to assess the antimicrobial activity of the tested compounds on MHA and SDA. In the solid agar medium, 8mm-diameter wells were made. 100 µL aliquots of every tested formulation were introduced into the wells. Using a metal caliper, the diameter of the zone of inhibition was measured and recorded in millimeters following a 24-hour stay in the

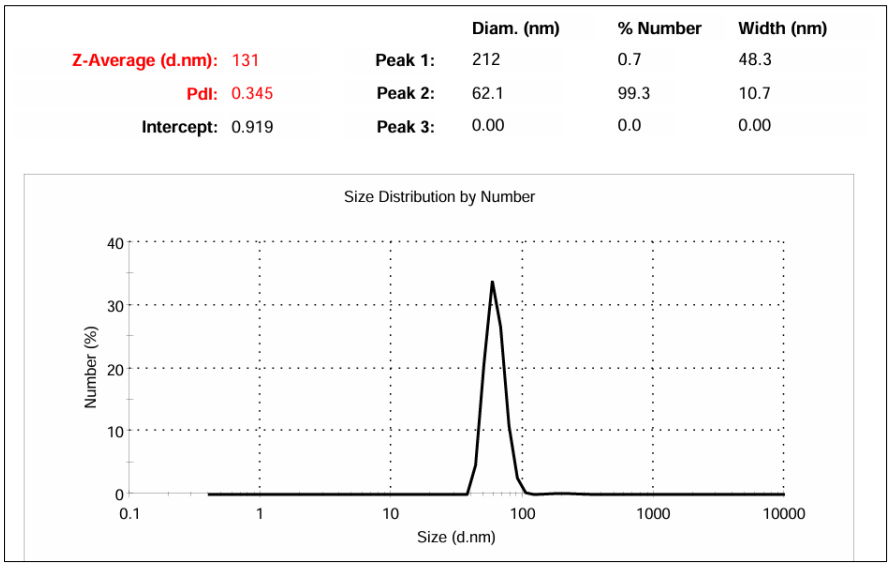

Fig. 1. The mean particle size (z-average diameter) and polydispersity index (PDI) of the GO-SLNs formulations.

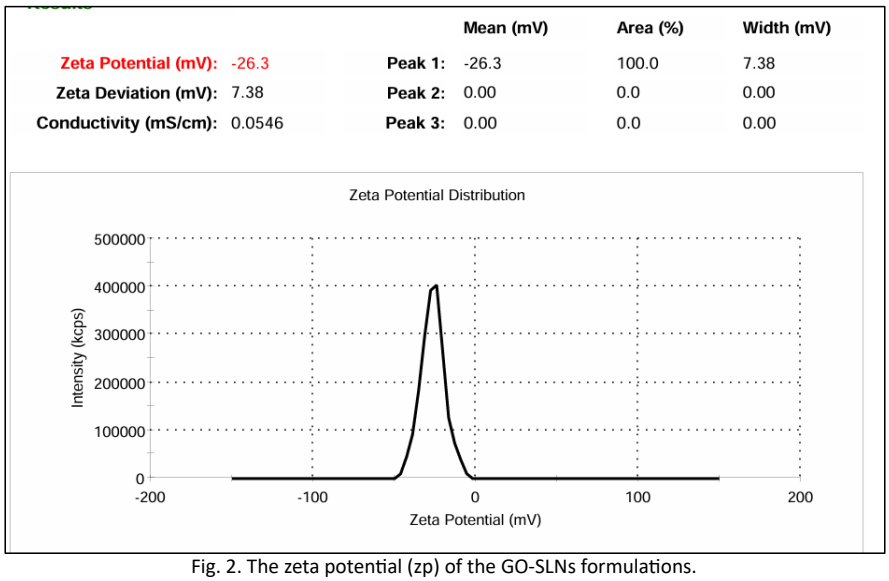

Fig. 2. The zeta potential (zp) of the GO-SLNs formulations.

incubator. The experiment was carried out three times for every bacterium and every Candida. The average zone of inhibition has been determined for each test GO-SLNs and the regular GO [34].

RESULTS AND DISCUSSION

Preparation and Characteristics of GO-SLNs

The particle size and its distribution are among the more significant factors related to quality, affecting other macroscopic characteristics. The formulation component, manufacturing techniques, and environmental factors (such as time, temperature, pressure, number of cycles, and equipment) all have an impact on the particle size of solid-liquid nanoparticles [35]. Based on the Z-average of GO-SLNs, the experiment's particle size was 110.5±3 nm, with a PDI of 0.320±0.04 and a zeta potential of -36.6±0.5 mV.

Determination of Encapsulation and Loading Efficiency

The most important criterion for evaluating the quality of SLNs at the end of the process is oil encapsulation efficiency (EE), expressed as % of the total amount of GO in the formulation. High oil encapsulation efficiency is a desirable quality in a nanocarrier. The findings indicate that encapsulation efficiency increased as GO concentration increased and was positively connected with GO quantity. Table 1's data showed that GO-SLNs had an encapsulation efficacy of 88±0.70% and a loading capacity of 0.56±0.0 [36,37].

Morphology Study

The SEM image of GO-SLNs is displayed in Fig. 1. The SEM scan revealed that the prepared SLNs had a spherical shape. Particle size distribution was similar to DLS. After loading, the nanocapsules had a spherical form, high dispersion, and a limited size distribution [31,37].

Differential Scanning Calorimetry (DSC)

From a technological and biopharmaceutical perspective, the physical condition of the particles is crucial. Lipid nanoparticles and other crystalline materials' melting and recrystallization behaviors can be better understood by DSC. Heating the sample to break down the crystal lattice provides detailed information on glass transition processes, eutectic mixtures, polymorphism, and crystal ordering 24. The use of differential scanning calorimetry was employed to examine the state of LNPs stabilized with poloxamer188. Differential scanning calorimetry thermograms of poloxamer188, gelatin microspheres, a combination of GO and gelatin microspheres, and the GO-SLN complex are displayed in Fig. 3. The GO-SLN complex's peak melting temperature (57.10°C) was found to be lower than that of commercial lipids (61.42°C) and greater than that of a gelatin microsphere and GO mixture (45.70 °C) [36].

In Vitro Release Kinetics of Garlic Oil in SLN

Because GO is highly liposoluble, the EE in all formulations was approximately 88%. However, it is difficult to be released from SLNs in a general medium that cannot provide a sink condition. The percentage of GO released from the GO-SLNs complex in the medium is displayed in Fig. 6. About 1% of the GO from SLNs was released as a result of the burst release that was seen within the first hour. 30% of the GO was released throughout the following 10 hours, demonstrating a prolonged release. DSC analysis of powdered

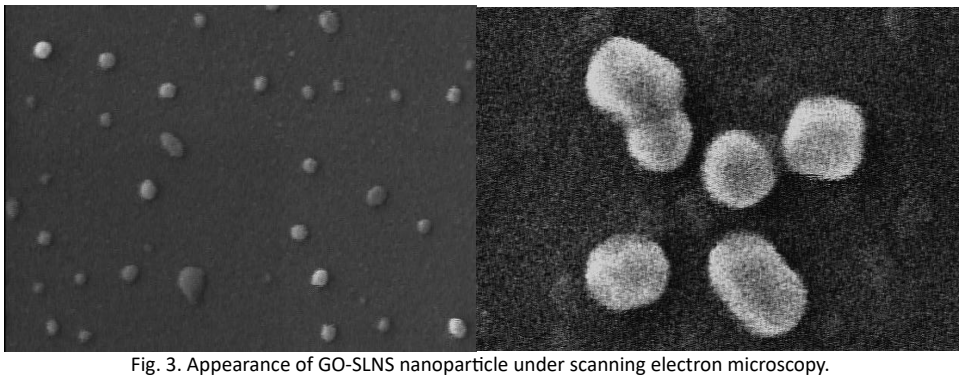

Fig. 3. Appearance of GO-SLNS nanoparticle under scanning electron microscopy.

SLNs revealed that GO dissolved, adsorbed on the surface of nanoparticles, or precipitated in the superficial lipid matrix; as a result, the dissolution profile of SLNs showed a burst of the drug during the initial stage. Gelatin microspheres in SLNs were also found to be preserved in a high crystal state. Drug release was steady and continuous in the later stage, suggesting that the drug's diffusion out of the rigid matrix structure governed the drug release rate. GO completely release from SLNs within 120 houes [33].

The Antimicrobial Study

Bacterial and fungal strains

The strains of the study (Gram-positive bacteria: *methicillin-resistant Staphylococcus*

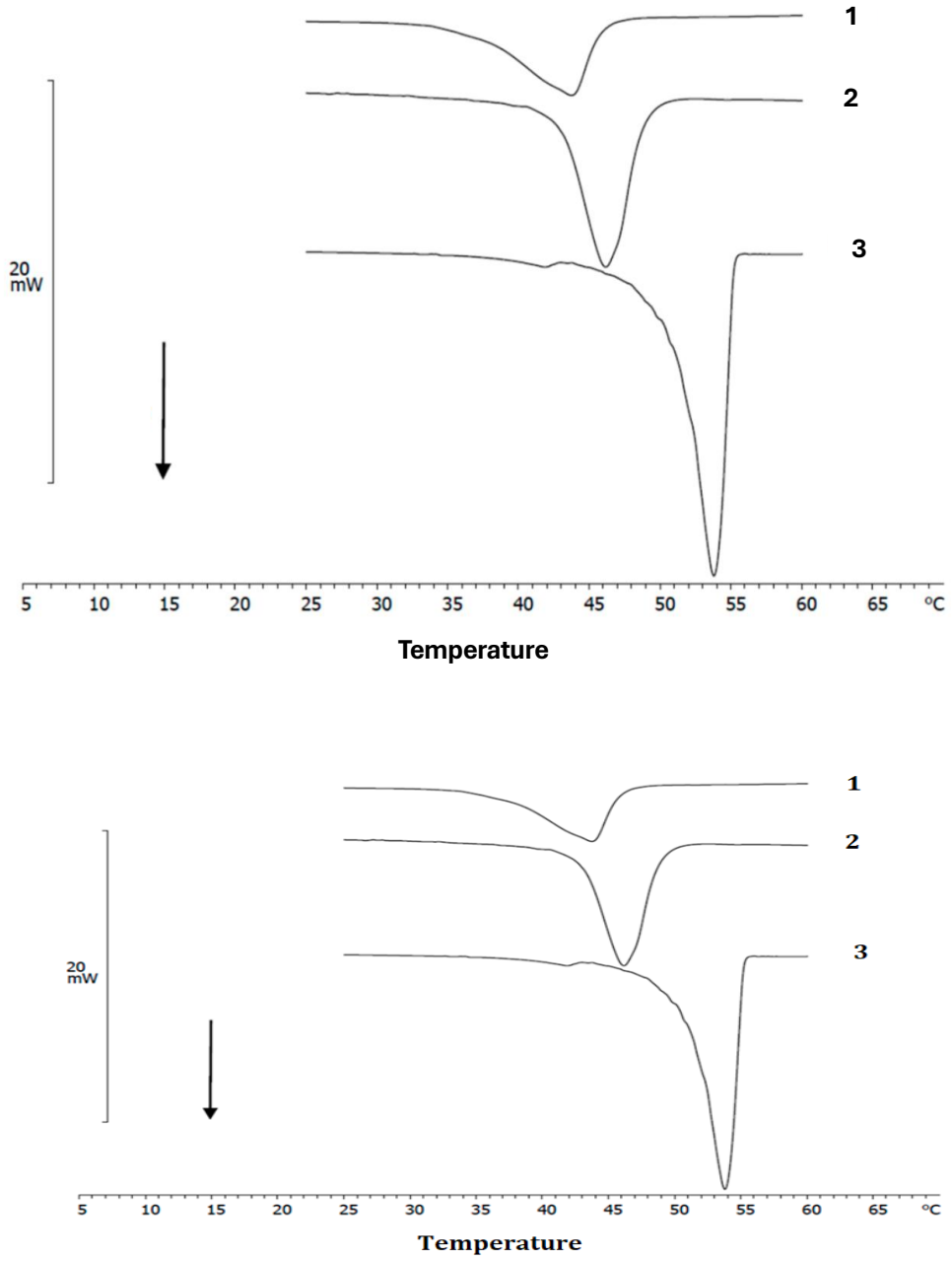

Fig. 4. Differential scanning calorimetry thermograms (DSC) of GO-SLNs dispersions heating from 30° to 100° C at a rate of 10° C. 1: Poloxamer 188; 2: physical mixture of GMS and GO; 3: GO-SLN complex.

aureus (*MRSA*), and Gram-negative bacteria: Escherichia coli and the fungus: *Candida albicans*) were provided by the Laboratory of Microbiology, College of Science at Wasit University.

The antimicrobial activity of GO-SLNs

The results of the antimicrobial activity indicated that the GO-SLNs had 74%, 54%, and 85% inhibition on the growth of *MRSA*, *E. coli*, and *C. albicans*, respectively. In addition, GO-SLNs exhibited more antibacterial activity against tested microorganisms than regular GO. The best antimicrobial efficacy of GO-SLNs was found in this study based on the measured zones of inhibition. It had the highest level of inhibition on the *MRSA* bacteria (36 mm), and (29 mm) against *C. albicans*, whereas the lowest level of antimicrobial activity was shown in *E. coli* (17 mm) (Table 1 and Figs. 6-8). According to the research, the GO-SLNs had the greatest effects on the bacteria under study [36].

According to these results, GO-SLNs inhibit the growth of the tested bacteria and fungi more effectively than regular GO alone, even at lower concentrations. This may be because the GO is efficiently delivered by the vehicle (SLNs) and interacts with the bacteria and fungi [38].

The anti-bacterial and antifungal activity of regular garlic oil was compared with the anti-bacterial activity of garlic oil-SLNs in the current study. Essential oils are natural, fragrant, volatile liquids that are derived from various plant sections and have applications in pharmaceutics and food science. Numerous investigations have established the high bioactivity of Essential oils, which includes antioxidant, antimutagenic, antiviral, anti-inflammatory, antifungal, antibacterial, and other diverse actions [39]. The primary issue with essential oil is its strong volatility and instability in the presence of light, air, and humidity. This can lead to easy evaporation and thus decreased efficiency [40]. SLNs are one of the new delivery systems that have been created to increase their stability.

Based on its unique organo-sulfur mixes, the garlic plant is widely used as a food flavoring and is believed to have a favorable effect on human health and well-being [41]. Diallyl disulfide, diallyl trisulfide, allyl propyl disulfide, disulfide, and most likely diallyl polysulfide are the primary

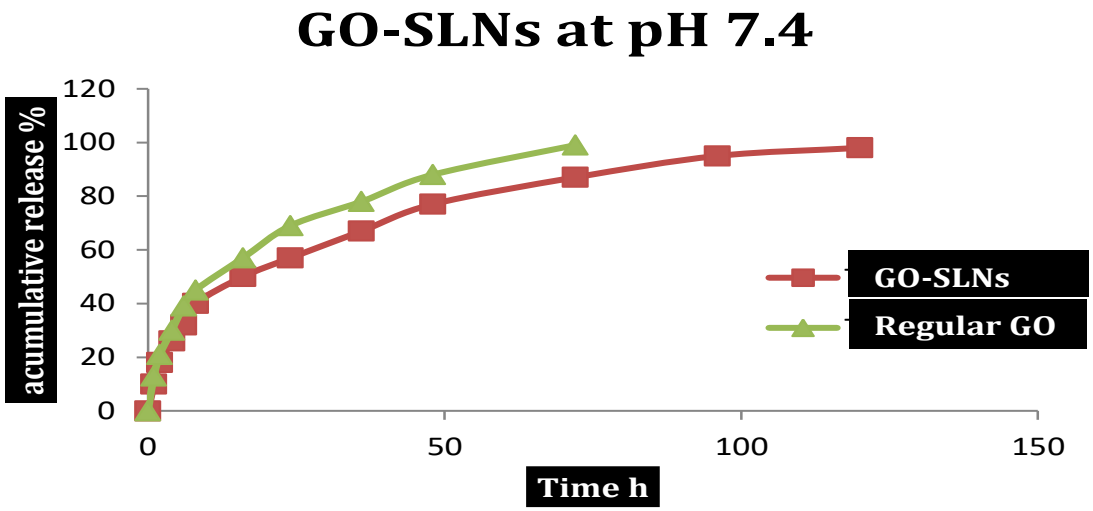

Fig. 5. Release profiles of GO-SLNs in comparison with Regular GO in dissolution medium at pH 7.4.

Table 1. Results of the study on the antimicrobial effects of GO-SLNs and Regular GO.

Species	Zone of inhibition (mm)		
	GO-SLNs	Regular GO	SLNs
MRSA	36	14	0
E. coli	29	11	0
C. albicans	17	9	0

components in garlic oil [42].

These chemicals have been shown to have antifungal, antibacterial, antiviral, and antiparasitic effects in previous studies [43, 44]. Furthermore, it has been observed that certain molecules, including diallyl trisulfide and diallyl disulfide, exhibit antibacterial activity [45].

Particularly, garlic (*Allium sativum* L.) essential oils have been discovered to exhibit beneficial anti-carcinogenic, anti-diabetic, and anti-microbial action. This is essential for a variety of medical and pharmacologic applications [46]. Moreover, these agents exhibit the capacity to lower blood pressure, cholesterol, and plasma aggregation [47].

High shear homogenization and ultrasound are dispersing techniques that were initially used for the production of SLN. For the preparation of SLNs, high-pressure homogenization has proven to be a dependable and effective technique [33].

We prepared and characterized GO-SLNs and assessed their efficacy on pathogens. Increased encapsulation of the active substance has been noted in certain investigations upon the application of GMS and Compritol 888 ATO in the lipid phase [48].

GMS and Compritol 888 ATO were used in this study as lipids. According to our results, loading essential oil in SLNs was 88±0.70%, and the loading capacity was 0.56±0.0in this formulation. Because of its strong encapsulation efficiency, GO is known to be a lipid-soluble molecule that disperses readily in lipid mixtures [49].

Numerous factors, such as the formulation chemical, production procedures, and environmental factors, influence the particle size of LNPs [50]. The zeta potential was -36.6±0.5 mV, the particle size average was less than 150 nm, and the PDI was approximately 0.320±0.04. These values can cause particle repulsion and prevent aggregations. The SLNs with zeta potential higher than ± 30 mV are normally considered physically stable [51].

A spherical particle shape and a particle size of about 100 nm are supported by the SEM image. The SEM pictures show that smooth-surfaced, spherical microparticles are formed as a result of the melt-high-pressure homogenization process [33].

To assess the degree of crystallinity, DSC studies were carried out (Fig. 2). DSC curves showed that the DSC pick of GO is not visible in GO-SLNs. These

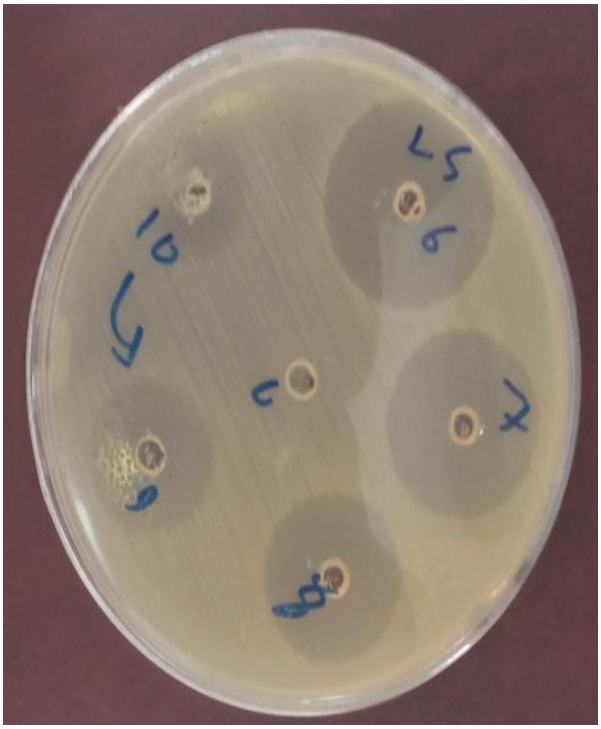

Fig. 6. Inhibition zone as shown by GO-SLNs and regular GO on MRSA.

results might indicate the oil incorporation and dissolution of the lipid matrix. It seems that SLNs stop GO from evaporating [36].

From a technological and biopharmaceutical perspective, the physical condition of the particles is essential. Lipid nanoparticles and other crystalline materials' melting and recrystallization behaviors can be better understood by DSC. Sample heating causes the crystal lattice to break down, revealing details on glass transition processes, eutectic mixtures, polymorphism, and crystal ordering [52]. Using DSC, the condition of lipid nanoparticles stabilized with tween and poloxamer188 was examined.

The SLNs have the best capacity for regulated release and defense against encapsulated essential oil due to their sphericity. This is because, in comparison to other forms of nanoparticles, the spherical shape has a long route for the flow of essential oil contained in the nanoparticles and the lowest contact surface with the aqueous medium of the dispersed phase [53].

The encapsulation efficiency in all formulations was higher than 80% due to the high liposolubility of GO. About 1% of the GO from SLN was released as a result of the burst release that was seen during the first hour. 10% of the GO was released throughout the following 30 hours, indicating a prolonged release. 1% of GO either precipitated in the superficial lipid matrix or adsorbed on the surface of NPs; as a result, the drug burst during the first stage of the SLNs' dissolution profile. Drug release was steady and continuous in the later stage, suggesting that the drug's diffusion out of the rigid matrix structure governed the drug release rate [33].

Due to their smaller size than cells, SLNs have the potential to enhance passive cellular absorption mechanisms, which would reduce transfer resistance and increase antibacterial action. According to Effert and Koch's findings [54], the pathogen's enzymatic degradation of the essential oil's active components is prevented by the nanocarriers. Conversely, SLNs protect essential oils from light, humidity, and acidity, among other environmental elements. Additionally, they easily increase the bioavailability of essential oils, produce more nano-sized carriers, and facilitate the regulated release of essential oils [55].

The level of SLNs in contact with microorganisms is noticeable, which helps to explain why the encapsulated essential oil-loaded formulations

Fig. 7. Inhibition zone as shown by GO-SLNs and regular GO on *E. Coli*.

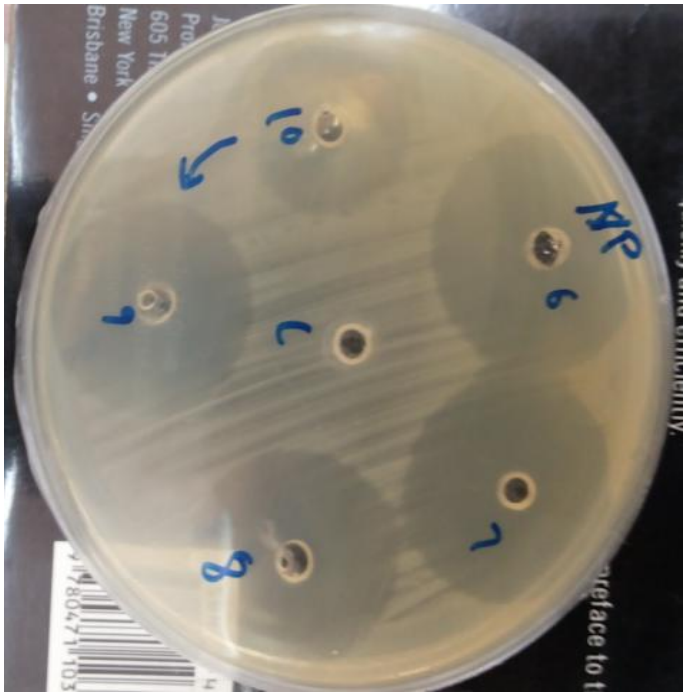

Fig. 8. Inhibition zone of GO-SLNs and regular GO on *C. albicans*.

are relatively more effective than the GO. SLNs can completely round the cell and cover its surface due to their bigger size than the microbe. Conversely, SLNs prevent the essential oil from evaporating. The encapsulation of the essential oil within these colloidal delivery methods causes a decrease in its rate of evaporation. It can therefore exhibit a bigger influence in comparison to the GO because it has a higher probability of coming into contact with microbes. Numerous additional studies suggested that SLNs are appropriate essential oil transporters, and SLNs have effectively included several essential oils [56].

The garlic oil nano-emulsion exhibited higher antibacterial activity against MRSA bacteria in contrast to E. coli. This could be explained by the fact that gram-negative bacteria are less sensitive to GO inhibition than gram-positive bacteria are. Compared to gram-negative bacteria, MRSA, a gram-positive bacterium, demonstrated a greater sensitivity to inhibitory activity [57].

CONCLUSION

According to our findings, it is possible to create GO-SLNs with the right size, drug loading, and release profile. In this experiment, the oil was added to a controlled-release nanoformulation to increase the antibacterial action of GO. GO is incorporated into SLNs via high-shear homogenization and ultrasonic techniques to shield its active ingredients from degradation and undesirable environmental factors. The produced nanocapsules demonstrated antimicrobial activity against the *fungus C. albicans* as well as all tested Gram-positive (*MRSA*) and Gram-negative (*E. coli*) bacteria. The developed compound (GO-SLNs) and regular GO under investigation both exhibited bacteriostatic action against *MRSA*. GO-SLNs showed better antimicrobial effects compared to regular GO. We may infer that the SLNs generated in this work were suitable carriers for the GO and represent an effective way to increase the essential oils' applicability as antimicrobials.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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