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Research Article

Formulation And Characterization of Nanolipid Carriers Loaded With Eugenol And Glycyrrhizic Acid: An In Vitro Evaluation Of Their Antimicrobial Potential

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Abstract:

Natural remedies are increasingly being used to cure and prevent infectious diseases, particularly those caused by multidrug-resistant (MDR) pathogens. These days, medication resistance is a worldwide issue. As a result, having medications on hand that can combat MDR infections is crucial. The spice clove (Syzygium aromaticum) is well-known for its many biological qualities. Eugenol is the main active ingredient in its essential oil (EO), which also contains other active compounds. However, eugenol's combined action with other components is responsible for the oil's biological effects. The purpose of this study is to examine how eugenol-glycyrrhizic acid extract nanolipid carriers (EGAE-NCL) can combat MDR bacteria that cause infections, illnesses, or problems in humans, including Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa. High shear force homogenization was used to create EGAE-NLC nanoparticles, which contained clove oil, licorice extract (glycyrrhizic acid), and clove extract (eugenol). SEM and HPLC were used to verify that EGAE-NLC nanoparticles were crystalline and pure. We concluded that the produced EGAE-NLC nanoparticles were crystalline in nature based on the SEM data. The average size of the EGAE-NLC nanoparticles was between 29 and 71 nm, and they demonstrated strong antibacterial activity. The research results demonstrated EGAE-NLC nanoparticles can inhibit the growth of MDR isolate S. aureus, P. aeruginosa and C. albicans.

1. Introduction

Antimicrobial resistance is a bacterium's capacity to withstand the effects of various antimicrobials. Microbes may become resistant to a medicine that was once effective against them in this kind of resistance [1]. Multidrug resistance (MDR) is the term used to describe this resistance to several medications. microorganisms exhibit a variety of resistance mechanisms, such as acquired resistance from other species, genetic mutation, or innate resistance in some microorganisms to a specific antibiotic [2]. Antibiotic resistance is expanding globally as a result of careless antimicrobial use. Resistance microorganisms are difficult to treat because they necessitate the use of different or stronger antibiotic dosages or the absence or scarcity of effective antibiotics. This is bad for countries at all stages of development. The World Health Organization (WHO) says that infections that are resistant to multiple drugs, also known termed "superbugs," are among the greatest threats to public health, killing millions of people annually all across the world [3]. With an emphasis on resistant gram-negative bacteria that pose the greatest threat to human health, the WHO published its list of priority diseases (antibiotic-resistance pathogens) [4]. According to the necessity of new antibiotics, the list is separated into three categories: critical, high, and medium priority. Acinetobacter baumannii, Enterobacteriaceae, and Pseudomonas aeruginosa are among the key group of multidrug-resistant bacteria that cause bloodstream infections and serious illnesses including pneumonia in hospitalized patients. One of the most prevalent potentially harmful bacteria,

Staphylococcus aureus, can inhabit many healthy carriers' sites asymptomatically. What a non-nasal transmission, especially in the oral cavity, contributes to the spread of antibiotic-resistant S. aureus strains in the medical field is poorly understood. Increased resistance to several kinds of first-line and last-resort antibiotics is a characteristic of the ESKAPE (Enterococcus faecium, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterobacter spp.) infections [5].

As an alternative to antibiotics, nanoscale particles (NPs) are being utilized more and more to treat bacterial infections. Antibiotic delivery systems, medicinal materials for wound healing and infection prevention, antimicrobial coatings for implantable devices, and bacterial detection for diagnostic purposes all make extensive use of NPs. Although little is known about antimicrobial mechanisms and their toxicity in real life, oxidative stress induction, metal ion release, and non-oxidative mechanisms are currently recognized methods. Because many simultaneous methods of action against germs would require multiple simultaneous gene mutations in the same bacterial cell for antimicrobial resistance to evolve, it is challenging for bacteria to develop resistance to metal nanoparticles [6,7].

Antimicrobial medication delivery has advanced significantly with the development of nanotechnology, especially nanoparticle engineering, and the growth of our understanding of infectious diseases. The development of different nanoparticle-based delivery platforms, such as liposomes, polymeric nanoparticles, dendrimers, and inorganic nanoparticles, has received a lot of attention. These nanoparticle techniques have demonstrated excellent results in the treatment and detection of bacterial infections by facilitating the targeted, responsive, and combinatorial administration of antibiotics, effective antimicrobial vaccination, and quick bacterial detection. The development of novel, unconventional treatments is required to treat bacterial infections that are resistant to antibiotics because to acquired resistance and/or the production of biofilms. Treatments based on nanomaterials hold promise for treating hard-to-treat bacterial infections since they can circumvent the current pathways linked to acquired drug resistance. In addition, the distinct size and physical properties of nanoparticles enable them to eliminate resistant diseases and target biofilms. Here, we describe the general methods by which nanomaterials target bacteria to manage infections linked to biofilms or acquired antibiotic resistance [3]. Because of their wide range of potential applications in numerous fields, nanomaterials have been the subject of extensive research. According to reports, surface effects and small size effects are imparted by the precise particle size of nanomaterials. As a result, nanoparticles have special qualities like photoelectric and thermomagnetic characteristics that set them apart from normal materials [8]. The second generation of lipid nanoparticles, known as nanostructured lipid carriers (NLC), were created to enhance transcellular penetration and drug absorption as well as a number of physicochemical characteristics, including high drug loading capacity, cost-effectiveness, ease of preparation, and thermal stability [9].

A plant that is grown in many nations is the clove (Syzygium aromaticum L.). Because of its anesthetic and analgesic properties, as well as its antibacterial, antifungal, and antioxidant properties, clove essential oil is utilized extensively. Its primary constituent, eugenol, which may be found in the oil at up to 90% concentrations, is largely responsible for these qualities [10]. Clove oil is classified as an essential oil. Once thought to be the "essence" of the plant from which they were extracted, many of these chemicals are now employed as flavorings and fragrances. Clove oil, which is made up of several components, is produced by steam distilling freshly powdered cloves. Eugenol, the main component, accounts for 85–90% [8].Eugenol is a volatile and bioactive naturally occurring phenolic monoterpenoid that belongs to the class of natural chemicals known as phenylpropanoids. It is typically found in a number of aromatic herbal plants, such as clove, tulsi, cinnamon, nutmeg, and pepper, despite being mostly isolated from the clove plant (Eugenia caryophyllata). Eugenol's numerous applications in a wide range of industries, including food, flavoring, cosmetics, pharmaceuticals, agriculture, and many more, are widely known. It is common knowledge of eugenol's pharmacological properties, which include its antibacterial, anticancer, antioxidant, anti-inflammatory, and analgesic effects. In medicine, a variety of eugenol derivatives are used as local anesthetics and antiseptics. Even though it is used for a lot of different things, eugenol has a lot of bad effects, especially if you take more than the recommended amount. It may result in convulsions, nausea, lightheadedness, and an accelerated heartbeat [11]. Clove has broad-spectrum antibacterial properties that work against a variety of pathogens, including bacteria, fungus, and even some viruses. The primary component responsible for these antibacterial properties is eugenol. Bacteria including Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli have

been demonstrated to be inhibited in their growth by it. Additionally, clove essential oil exhibits antifungal properties against a variety of fungal species, such as Trichophyton rubrum, Aspergillus Niger, and Candida albicans. Cloves include eugenol, which has antibacterial qualities that help fight dental infections and lessen foul breath. Because of its antimicrobial properties, clove oil is frequently found in mouthwashes and dental care products [12].

The underground creeping stems of growing licorice (Glycyrrhiza glabra L.), a well-known commercial herb, can be found in southern Europe and eastern Asia. The food, tobacco, pharmaceutical, and cosmetic sectors all make extensive use of the plant's subterranean extract. Applications of licorice in the food business include chewing gum, confections, and the production of a variety of beverages. Numerous medical properties, such as its anti-inflammatory, anti-cancer, anti-oxidant, anti-ulcer, hepatoprotective, memory-enhancing, hypocholesterolemic, and anti-depressant properties [13]. have been investigated in relation to licorice. Glycyrrhizic acid (glycyrrhizin) is a glucosiduronide derivative of 3beta-hydroxy-11-oxoolean-12-en-30-oic acid ($C_{42}H_{62}O_{16}$) with an amphiphilic structure that is widely used as a sweetener in foods, candies, and other confections. The GA content, a significant index listed in the most significant worldwide pharmacopeia, is used to evaluate the underground portions of the plant employed in various preparations and goods. GA has been shown to have hepatoprotective, antiviral, anti-inflammatory, and anticancer properties.

Additionally, Esmaeili et al. (2024) demonstrated that GA may have therapeutic benefits for liver and skin conditions [12].

This study aims to prepare and investigate the role of eugenol-glycyrrhizic acid extract nanolipid carriers (EGAE) nanolipid carriers to concentration of MDR microorganisms responsible for human disorders, diseases, or infections, such as Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans.

2. Materials and Methods

2.1 Green methods of EGAE-NLCs synthesis

A Clove (Syzygium aromaticum) and Licorice (Glycyrrhiza glabra) were collected from the local Aera in Baghdad, the plants were kept at room temperature until use, and plants were then classified by College of Science Herbarium / University of Baghdad.

2.2 Preparation and Identification of Eugenol Extract (EE)

A modified method for eugenol extraction were used (13). One-hundred grams of clove was grinding with mortar then add 500 ml ethanol (99.5 %) – water (30:70) for 1 hrs. at 50°C with stirrer after one hour let the mix cool and filter, take 5 ml of filtered and dilute to 50 ml with water (milky-cloudy solution). The solution was transferred to a separating funnel and extracted with chloroform take the aqueous layer and evaporate by hot plate [14] Identification and estimation of EE by HPLC technique. Using a Cosmosil C18 analytical column (150 mm ×4.6 mm × 5 m) kept at room temperature, chromatography separation for the analyte was accomplished. The mobile phase was pumped at a rate of 1 mL/min. Before being used, the mobile phase was degassed in an ultrasonic bath and filtered through a 0.45 m nylon membrane filter. A chromatographic peak was found at 215 nm, the injection volume was 30 mL, and the flow rate was 1.0 mL/min [15].

2.3 Preparation and Identification of Glycyrrhizic Acid Extract (GAE)

Modified method for glycyrrhizic acid extraction 500 mg ethanol: water (70:30) was used to extract a well-ground powder from the dried root of one sample [16]. Thus, after adding the solvents to the powder, it was sonicated for 30 minutes in an ultrasonic bath and stirred for 5 hours at 60 °C. The extracts were centrifuged and fed into the HPLC after going through the filter after being allowed to cool to room temperature [12]. They utilized a 4.6 mm, 250 mm, 5.0 m, Waters, USA, X Bridge C18 column. The mobile phase had a flow rate of 0.8 mL/min and included 7% acetic acid and acetonitrile (60:40, v/v). The injection volume was 20 _L for each sample. At a wavelength of 254 nm, glycyrrhizic acid (GA) was observed. The GA summit was located by evaluating its retention duration against standards, and the calibration curves were used to determine the concentration [17].

2.4 Preparation of EGAE-NLCs

Similar high shear force homogenization techniques were employed, albeit with minor modifications to the production process [17]. The EGAE was first weighed and dissolved in 2 milliliters of D.W. A magnetic stirrer was used to thoroughly dissolve 500 mg of Tween 80 (an aqueous surfactant) in 25 mL of distilled water, which was then heated to 50 °C to create the aqueous phase. A water bath was

used to heat the lipid phase, which contained 220 mg of liquid lipid and 330 mg of solid lipid (coconut butter), to 50 °C (5 °C over the melting point of C.B.). A high shear homogenizer (Heidolph, Germany) and 250 µl of the solution were then applied to the lipid phase. used a sampler to gradually introduce water and EGAE to the lipid phase while it was being sheared. The lipid phase was then gradually supplemented with the aqueous phase. The resultant emulsion was agitated for an additional 20 minutes at 20,000 rpm in a homogenizer. In order to further reduce the particle size to the nanoscale and distribute it more evenly, it was subsequently moved to a probe sonicator (QSonica Q500, USA). In order to generate irregular lipid crystals, the system was finally chilled [18].

2.5 Analysis of the zeta potential and particle size

The samples were diluted with D.W. twenty times, and the mean particle size (z-average size), polydispersity index (PDI), and zeta potential of the samples were measured in three replications at room temperature (22 °C) using dynamic light scattering (DLS) and a particle size analyzer (Zeta Sizer ultra model, Malvern Instruments Ltd., Worcester, Worcestershire, UK). The samples were diluted twenty times before the DLS data were recorded, and the volume diameter was used to determine the average particle size.

The zeta potential is a method for determining a particle's surface charge in a liquid. The physicochemical properties of the drug, polymer, vehicle, electrolyte presence, and adsorption all affect the value of the zeta potential, which is used to predict dispersion stability. The Malvern Zeta sizer is used to measure it. Zeta potential is measured by diluting a nanoemulsion and estimating its value based on the electrophoretic mobility of oil droplets. According to Changediya et al., a zeta potential of less than 30 mV is thought to be sufficient to ensure the nanoemulsion's physical stability [18-20]. Morphology of EGAE -NLCs: Scanning electron microscopy (SEM)

Thermo Fisher Scientific's SEM was used to examine the surface morphology of samples that had been diluted 20 times with D.W. A thin layer was created on a sterilized lamella by moderately dispersing one or two drops of the diluted material. The lamella was dried and ready for SEM analysis in an incubator set at 45 °C. To determine the sample conductivity prior to viewing, the sample was placed on a brass stub and covered with gold. A 15 kV accelerating voltage was used for SEM [19].

2.6 Biological efficacy of EGAE-NLCs

The effectiveness of EGAE -NLC synthesized, was evaluated against selected clinical isolates of Gram-negative bacteria MDR P. aeruginosa and Gram-positive bacteria Methicillin-resistant Staphylococcus aureus (MRSA) and MDR yeast C. albicans for antimicrobial activity. These selected isolates were diagnosis and confirmed by Al.joubori et. al., [21]. The assessment of antimicrobial activity utilized the agar well diffusion method, wherein sterilized Petri plates containing 30 mL of Mueller Hinton agar medium were employed for bacterial isolates and sabouraud dextrose agar for yeast isolate [22]. For the purpose of the evaluation, wells with diameters of 6 mm and concentrations of 100, 75, 50, and 25% were designated as wells A, B, and C, with well D serving as a control. Each well contained 40 L of biosynthesized EGAE-NLCs (5 mM). Well D was not filled as negative control, the mean zone of inhibition (ZOI; mm) was measured in mm for all surrounding wells after a 24 h incubation at 37°C [17].

3. Results and Discussion

3.1 Identification and estimation of Eugenol extract (EE)

The amounts of eugenol using HPLC technique in accordance with eugenol standards are displayed in Figure 1.

To measure the concentration of eugenol, Shafira et al. estimated it using HPLC-UV analysis [13]. The average eugenol concentration of the total oil extracted was greater, according to the results for ethanol 90%.

The correlation coefficient (R2) determined by KANWAL et al. using HPLC was 0.973531. Quantitative estimation revealed 740 parts per million of eugenol. These characteristics demonstrated clove's value as a spice in the agro-food and pharmaceutical industries [23].

3.2 Identification and estimation of Glycyrrhizic Acid extract (GAE)

The concentrations of GAE using HPLC technique in accordance with GA standards are displayed in Figure 2. The HPLC-UV method used by Brovchenko et al. to quantitatively determine GA in commercial licorice roots was evaluated for accuracy, precision (both intralaboratory and convergence), linearity, and specificity [24].

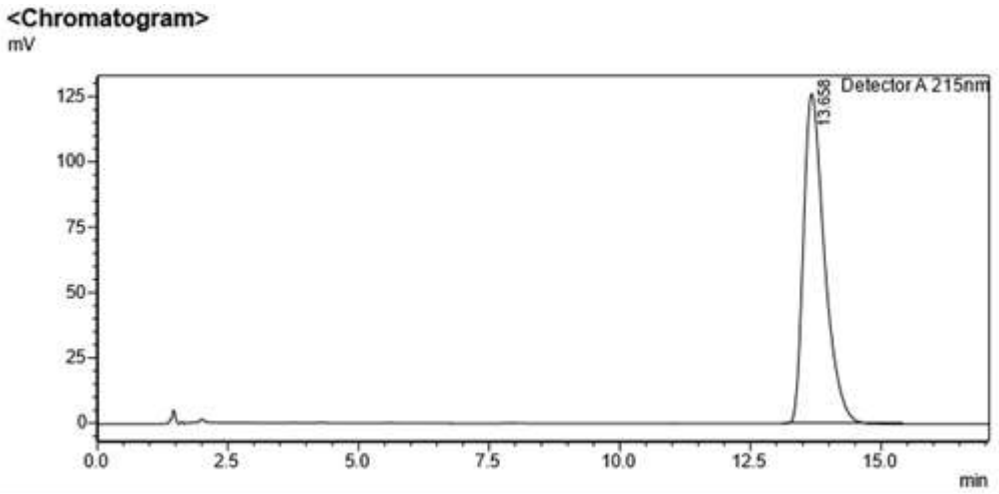

Figure 1. *HPLC chromatogram of Eugenol*

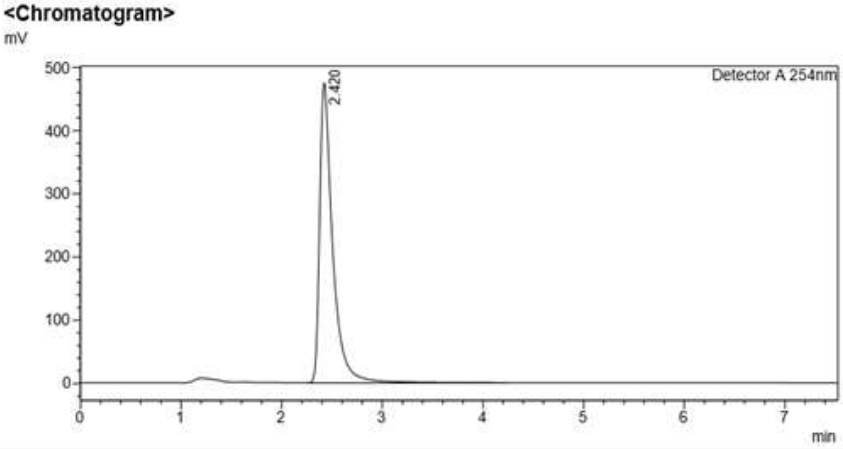

Figure 2. *HPLC chromatogram of glycyrrhizic acid*

In contrast, Kurkin et al. were created to use HPLC to quantify the amount of GA and licurazide present in licorice roots [25]. the mistake in calculating the 95% confidence level of licurazide and GA in the licorice roots. Glycyrrhizic acid (GA), the primary licorice component, was identified at 254 nm and in typical HPLC chromatograms in another investigation [17].

3.3 Scanning Electron Microscopy (SEM)

Figure 3 depicts the EGAE-NLCs' surface morphology. The crystalline structure of lipids showed the distribution of the nanoparticles. The complex's creation made the EGAE particles invisible. SEM revealed round particles. Additionally, the particle size's narrow distribution was validated by its uniformity. The particles were less than 100 nanometers. In microscopic investigations of nanoparticles containing essential cardamom oil, Keivani et al. discovered spherical particles with a smooth surface and lower particle size than the DLS particle size [26]. Azar et al. demonstrate the OLE-NLC's surface shape and the nanoparticle distribution in the lipid crystal structure. Because of the complex's creation, OLE particles [18].

3.4 Particle size, PDI and zeta potential

Figure 4 displays the formulations' mean diameter, PDI, and zeta potential. The formulations' mean size was less than 100 nm. Out of all the formulations, NLCs were the smallest (50 nm). The formulation's average size fell between 29 and 80.3 nm. The generated NLCs' PDI values, as displayed in Figure 5, ranged from 0.250 to 0.370, suggesting

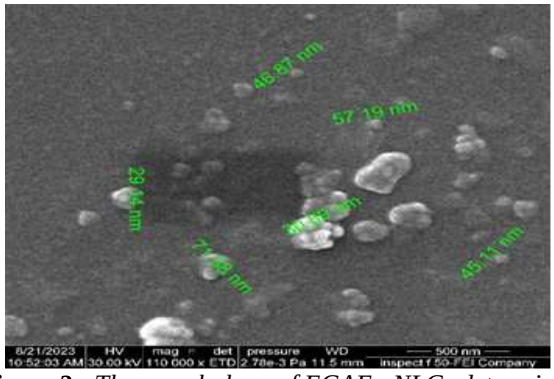

Figure 3.*: The morphology of EGAE –NLCs determined by SEM*

a comparatively broad size distribution. Good compatibility between liquid lipids and CEO as a bioactive material is confirmed by the tiny size of produced NLC systems. Smaller particles are typically produced as liquid lipid's viscosity drops. Reduced particle size will result in increased solution clarity, colloidal stability, and specific surface area, all of which will raise Figure 4 displays the formulations' mean diameter, PDI, and zeta potential. The formulations' mean size was less than 100 nm. Out of all the formulations, NLCs were the smallest (50 nm). The formulation's average size fell between 29 and 80.3 nm. The generated NLCs' PDI values, as displayed in Figure 5, ranged from 0.250 to 0.370, suggesting a comparatively broad size distribution. Good compatibility between liquid lipids and CEO as a bioactive material is confirmed by the tiny size of produced NLC systems. Smaller particles are typically produced as liquid lipid's viscosity drops. Reduced particle size will result in increased solution clarity, colloidal stability, and specific surface area, all of which will raise [25].

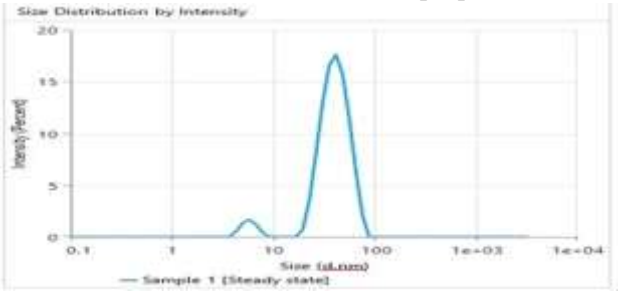

Figure 4. *Particle Size of EGAE –NLCs*

In the absence of other inhibitory variables like high viscosity and steric hindrance, colloids containing particles with low zeta potential (positive or negative) will be extremely prone to coagulation and aggregation. The zeta potential of the EGAE-NLCs was -35 mV. In suspensions with a zeta potential greater than 30 mV, aggregation is [27]. Greater stability of the nanocarriers is caused by a higher zeta potential because it increases the repulsive force between nanoparticles [28]. Therefore, the long-term stability of nanoparticles may be significantly influenced by electrostatic repulsion [29]. When producing NLC systems with vegetable oils, Manea et al. found that the zeta potential ranged from -30 to -58. Systems with Tween 80 had a lower zeta potential than systems without Tween 80 [30]. According to Azar et al., (2021) the hydrogel particles that were produced and contained OLE-NLC had a zeta potential of -23.9 mv, which indicated that they were negatively charged and had a lower zeta potential than the OLE-NLC particles. Because the pectin displayed its negative charge, it's possible that the interaction between the OH groups of oleuropein and the amine groups of sodium caseinate in the NLC surfactant layer neutralized the sodium caseinate's positive charge [18].

3.5 Antimicrobial activity of EGAE-NLCs

As illustrated in Table 1, the results demonstrated that the producer of EGAE-NLCs via eugenol and glycyrrhizic acid extracts at all concentrations were actively targeting specific clinical isolates of Gram-positive bacteria Methicillin-resistant Staphylococcus aureus (MRSA) and Gram-negative bacteria MDR P. aeruginosa as well as MDR yeast C. albicans. At 100 mg/ml, EGAE-NLC

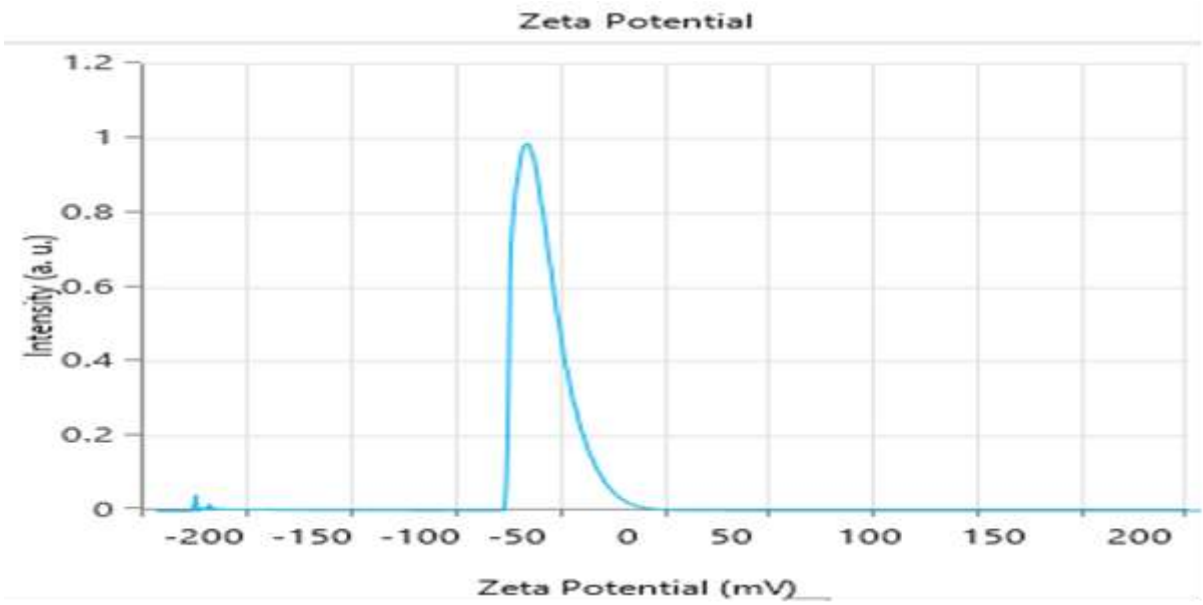

Figure 5. *Zeta potential of EGAE –NLCs*

demonstrated S. auras, and the extract's zone of inhibition (ZOI) measured 24 mm. The extract for P. aeruginosa displayed a ZOI of 26 mm. After treatment, C. albicans showed a zone of inhibition measuring 25.9 mm. Bai et al. claimed that eugenol may effectively inhibit the growth of S. aureus [31], At a dose of 1000 µg/mL, eugenol has been found to inhibit P. aeruginosa growth (NEJAD et al.). The full inhibitory action against these bacteria is demonstrated at 2000 µg/mL [32]. Sawatphakdee et al. demonstrate that clove oil inhibits S. aureus growth more effectively than the tested drugs [33]. The antibacterial efficacy of AgNPs derived from S. aromaticum leaf extract at 50 mg/mL was evaluated in another investigation, revealing a 25 mm and 23 mm zone of inhibition against P. aeruginosa [34]. Using eugenol extract, da Silva et al. demonstrated that the inhibition zones against E. coli, P. aeruginosa, S. aureus, and C. albicans had diameters of 13.70.8 mm, 11.50.5 mm, and 10.80.8 mm, respectively, in a different study [35], while eugenol was active (inhibition halo 12) [36], against P. aeruginosa. Research showed that eugenol had antibacterial efficacy against S. aureus strains, displaying inhibitory halos that measured 7.75 mm in diameter [37]. According to Rodino et al. [38], the majority of GAE's pharmacological effects also include antimicrobial activity, which inhibits bacterial infection by reducing bacterial growth, gene expression, and microbial toxins produced. Long et al. demonstrate that GA inhibits the growth of methicillin-resistant Staphylococcus aureus (MRSA) both in vitro and in vivo by lowering the expression of important staphylococcal virulence factors like hla and saeRS. [39]. Significant antibacterial activity was demonstrated by the alcoholic extract from G. glabra roots, which developed areas of growth inhibition against B. cereus, P. fluorescens, E. coli, and S. aureus [40]. Another study that used GA extract the impact of GA on Pseudomonas aeruginosa was examined by Yoshida et al. [38]. In addition to providing perspectives on the development of versatile nanoplatforms to overcome some limiting physicochemical properties and to enhance the therapeutic benefits of GA, Nascimento and Araújo summarize the pharmacological activities of GA and its beneficial effects against various health problems [40]. The hydrothermal approach was used to synthesis GA-NPs, as demonstrated by Rijo et al. The spherical shape of GA-NPs with a diameter of 50 nm was validated by characterization, demonstrating the high bactericidal efficacy of GA nanoparticles against MRSA and S. aureus [41]. The bactericidal effect of derivatives of glycyrrhizic acid has been emphasized in earlier investigations. At high concentrations (above 62.5 mg/L), GRA was shown to be effective against [42]. According to the current findings, generated EGAE-NLCs efficiently inhibited the development of S. aureus, P. aeruginosa, and C. albicans. As a result, they may be used as a natural antibacterial agent to control diseases caused by these three pathogens. The zones of inhibition seen against different pathogens demonstrate, in conclusion, that the EGAE-NLC solution has antibacterial and antifungal qualities. Although the plant extract's efficacy differed depending on the pathogen, it generally showed strong antibacterial activity.

Table 1. *Antimicrobial activity of EGAE–NLCs against pathogenic microorganisms*

EGAE-NLCs Concentration	**Zone of inhibition (ZOI) (mm)**		
	MRSA	**MDR *P. aeruginosa***	**MDR *C. albicans***
25 mg/ ml	14 mm	17 mm	16 mm
50 mg / ml	19 mm	21 mm	18 mm
75 mg/ml	21mm	23 mm	22 mm
100 mg/ml	24 mm	26 mm	25.9 mm

4. Conclusion

Using the hot-high shear homogenization process, EE and GAE were encapsulated in nanolipid carriers (EGAE-NLCs) in this study. HPLC analysis revealed a high concentration of the active ingredient in the nano-emulsion. This technique worked well for producing EGAE-NLC, which reduces particle size to less than 100 nm at the nanoscale. When EGAE-NLCs' antibacterial ability was evaluated in vitro, it clearly inhibited the growth of multidrug-resistant isolates (MRSA, MDR P. aeruginosa, and MDR C. albicans) following a 24-hour incubation period at varying concentrations. MRSA was detected by EGAE-NLCs at 100 mg/ml, and the extract showed a zone of inhibition (ZOI) of 24 mm. The extract for MDR P. aeruginosa displayed a ZOI of 26 mm. The zone of inhibition for MDR C. albicans measured 25.9 mm. Reported works detailed about some nano particles in the literature [43-47].

Author Statements:

- **Ethical approval:** The University of Baghdad Ethical Committee approved the project after a series of scientific and administrative procedures.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper

- **Acknowledgement:** The cooperation of the Postgraduate Studies Laboratory, Department of Pharmaceutics, College of Pharmacy, University of Baghdad is appreciated.

- **Author contributions:** M.T. Al. Musawi proposed the topic of research and guidance and the review and proofreading the research for antimicrobial study. K.K. AL-Kinani suggested the preparation and characterization of nano lipid carriers as the subject of research, guidance, and a review and proofreading of existing research. Y. S. Al. Jubouri prepared the samples and analyzed parameters and writing, as well as the publishing process.

- **Funding information:** The authors declare that there is no funding to be acknowledged.

- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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