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RESEARCH PAPER

Environmentally Friendly Silver Nanoparticle Synthesis Employing *Nigella sativa L.* Essential Oil and Investigating Their Antibacterial Potential

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ABSTRACT

In this study we extract the essential oils of *Nigella sativa L.* seeds that taken from Iraqi market using Clevenger apparatus, Gas Chromatography-Mass Spectroscopic (GC-MS) used to determine the components, A total of thirty compounds were identified by their peak values, retention time, and molecular mass. the major phytoconstituents such as Z, Z-6,13-Octadecadien-1-ol acetate (26.49%), Octadeca-9,12-dien-1-ol (21.74%), and 9,12-Octadecadienoic acid methyl ester (15.01%) were identified by high peak values. The FT-IR spectrum analysis was also carried out, and the results confirmed the presence of functional groups such as amines, alkanes, acids, esters, alkyl and alkenes. Silver nanoparticles (AgNPs) with Nigella arvensis L. essential oils prepared, An intense surface plasmon resonance band at 431 nm, which indicates the production of nanoparticles, was used to characterize AgNPs using UV–vis absorption spectroscopy. Silver's connection with essential oils is indicated by the shift of the active group peaks, as demonstrated by Fourier transmission infrared spectroscopy (FT-IR). Field Emission Scanning Electron Microscopy (FESEM) revealed 35.9 nm-sized silver nanoparticles. The X-ray diffraction spectrum (XRD) pattern unequivocally shows that the AgNPs produced in this synthesis were crystalline. Zeta potential measurements, which reached -26.3, demonstrated the stability of silver nanoparticles. Using the Minimum Inhibitory Concentration (MIC) test, it was discovered that Nigella sativa L. essential oil was more effective against Enterococcus faecalis. The concentration of silver essential oil nanoparticles AgEO (A2) was 0.16 mg/mL, whereas the MIC of the only essential oil was 2.5 mg/mL.

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INTRODUCTION

Nigella sativa (Black Cumin) seeds come from an annual herbaceous plant in the Ranunculaceae family. The black, fragrant seeds are traditionally used as a garnish for many dishes (meat, bread) in the Middle East, Egypt, and India. In addition, black cumin seeds are used as a substitute for pepper due to their gentle effect on the digestive system [1]. In addition, they are used to treat respiratory and digestive diseases, headaches, fever, and rheumatism [2]. NS seeds contain 30-40% fatty oil, 20-30% protein, 3.7-4.7% ash, and

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25-40% total carbohydrates, including antioxidant lignans such as saponin and melanthin [3]. This percentage varies depending on the time, location and harvesting method. The oil fraction contains a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodaitic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic and stearic acids) account for about 30% [4]. In addition, the oil contains alkaloids, phytosterols, tocopherols, saponins, flavonoids and finally essential oils (EOs) (0.4-2.5%) [5]. Numerous active principles of NSEOs have been isolated, identified and described in numerous experiments. The percentage of active ingredients varies, but the main active ingredients are thymoquinone (TQ), p-cymene, carvacrol, sesquiterpenes longifolene, trans-anethole, 4-terpineol, thymol, and α-pinene. Research has shown that some of these substances may have significant effects on human health [6]. NS has been shown to have antibacterial [7–9], antioxidant [10–14], and antitumor properties [15]. However, in recent years, several articles have been published showing that NS oil or extract can modulate immune responses in various diseases associated with hypersensitivity reactions, such as asthma, allergic rhinitis, and rheumatoid arthritis. For example, one clinical study showed that asthma patients who were given boiled NS extract showed a reduction in symptoms of the disease, such as attack frequency and wheezing, at subsequent consultations [16].

MATERIALS AND METHODS

Essential oils

Extraction of Essential Oil

The Clevenger apparatus was used to extract the oil from fresh plant material. 250 g of *Nigella sativa* seeds were placed in a 1000 mL flask containing 750 mL of distilled water. The entire solution was then boiled for 3 h to maximize the extraction of the essential oil. The yield of essential oil was calculated using the following formula:

$$\text{Yield} = \frac{\text{m}\prime}{\text{m}} \times 100\%$$

Where *m'* is the mass of essential oils obtained in grams; *m* is the mass of plant material in grams. The amount of extracted essential oils was 5.2g so the yield will be 0.69.l

Gas chromatography

Phytochemical characterization of the essential oils was performed by GC-MS using a non-polar silica column. The analysis was performed under the following conditions: initial temperature of 40°C/2 min, rate of 2°C/min, final temperature and injector temperature set at 260°C/10 min and 250°C, respectively. Helium was used as the medium (1 mL/min) and injected in "split" mode for this analysis. The ionization energy and ion source temperature were 70 eV and 200 °C, respectively, and the scan mass range was m/z 20–500. The oil was diluted in n-hexane solvent (10:100) before injecting 1 µL. Chemical identification was based on retention index (RI) and comparison with the ADAMS database.

Essential oils Silver Nanoparticles

Synthesis Nanoparticles

5 mL (5% v/v) of aqueous black seed extract was added to 99 mL of 10 mM (1.698 g) aqueous $AgNO_3$ solution in a round-bottom flask. The flask had a magnetic bar and a condenser attached. For three hours, the mixture was kept at 85 °C and stirred constantly. After a while, the solution's hue changed from pale yellow to a reddish brown, signifying the development of nanoparticles. The reaction then ceased after three hours, and pure Ag NPs were separated by centrifuging the resultant mixture at 9,000 rpm and periodically washing it with distilled water.

In order to eliminate the unbound free residual extract entities from the Ag NPs' surface, the produced Ag NPs were suspended in DI water, agitated for a few minutes, and then centrifuged for 30 minutes at 9000 rpm. To guarantee that the Ag NPs were better purified, this procedure was carried out many times.

Characterization of Silver Nanoparticles

A Shimadzu UV-1800 UV-Vis spectrophotometer was used to record the best measurements of the produced Ag NPs at various time intervals. The wavelength scale was set between 190 and 990 nm, and they were carried out in quartz cuvettes. An Ultima IV X-ray powder diffractometer (Rigaku, Tokyo, Japan) was used to measure X-ray powder diffraction (XRD) with Cu Kα radiation (λ = 1.5418 Å). The JEM 2100F (JEOL, Tokyo, Japan). For TEM measurements, an accelerating voltage of 200 kV was employed. A Perkin-Elmer 1000 infrared (IR)

spectrometer (Waltham, MA, USA) was used to conduct FT-IR measurements.

Antibacterial study

Strains of bacteria

Studied bacteria *Enterococcus faecalis* was isolated from clinical specimens of patients with diabetic foot ulcers admitted to the Misan Provincial Diabetes and Endocrinology Center. Based on the colony characteristics, bacterial culture and biochemical data of the isolate (*E. faecium*), it was identified by biochemical tests according to standard microbiological procedures (1,2). A single colony of the strain was picked and suspended in a 5mL test tube containing Müller-Hinton broth (MHB) and cultured at 37°C for 6 hours until the McFarland standard turbidity reached 0.5. Before use, the culture was diluted 1:10 with Müller-Hinton broth.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) were determined by the microdilution technique in 96-well round-bottom microplates, using MTT as an indicator of viability. A series of half dilutions of the essential oil (10, 5, 2.5, and up to 0.04) ppm were prepared. The dilution method consisted of making a series of wells with Mollar-Hinton broth (MHB). The wells were filled with 90 µL of MHB (added to each well except row 10). 90 µl of the essential oil was then added to the first well and diluted. Each well (except rows 10 and 12) was then inoculated with 10 µl of bacterial suspension (108 CFU/ml). The wells in row 10 contained only the essential oil, row 11 contained only the bacteria, and row 12 contained only the culture medium. The microplates were incubated at 37°C for 24 h. To determine the MIC, 25 µL of MTT 5 mg/mL (w/v) was added to each well and then incubated for 30 min at 30 °C. The MIC was defined as the lowest oil concentration that did not induce a color change in MTT and corresponded to the absence of bacterial growth [3,4].

Determination of Minimum Bactericidal Concentration (MBC)

The MBC is calculated based on 3 µL wells showing no bacterial growth on solid media after inoculation and incubation at 37 °C for 24-48 hours. The MBC is considered the lowest concentration of essential oil at which no bacterial growth is observed [5].

RESULTS AND DISCUSSION

GC analysis

The species, the environment, the portions of the plant that are taken, the season, and other variables can all affect the chemical makeup of essential oils, which are secondary plant metabolites [6].

In this work Gas Chromatography-Mass Spectroscopic (GC-MS) was used to determine the bioactive components found 30 compounds in the

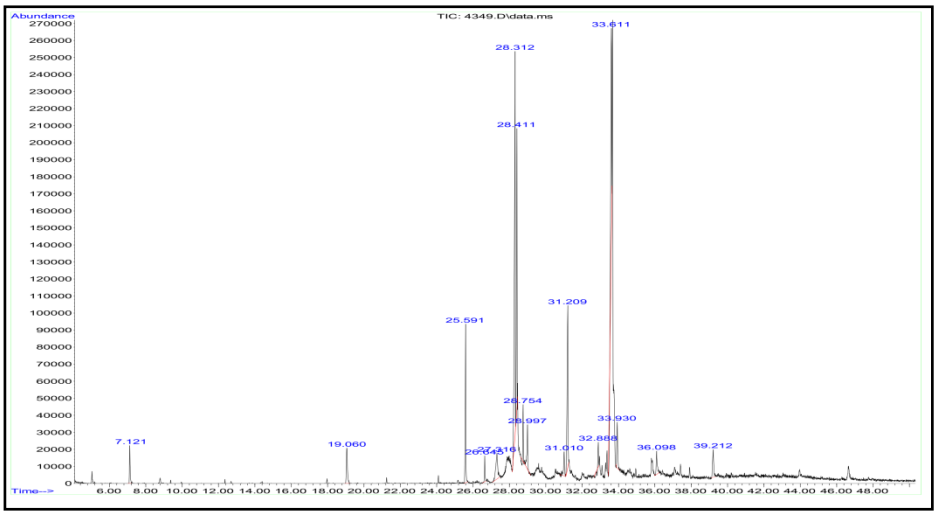

Fig. 1. Show GC-mass of extracted EO's.

Table 1. Show Essential oils compound and its percent.

No.	Name	CAS Number	RT (min)	Area %
1	Benzene, 1-methyl-2-(1-methylethyl)-	000527-84-4	7.122	1.19
2	1-Butene, 4-cyclopropyl-	007736-35-8	8.788	0.18
3	2,3,6-Trimethylbicyclo[4.1.0]hept-2-ene	000000-00-0	12.358	0.07
4	2-Furanmethanol	000098-00-0	12.69	0.04
5	Propanenitrile	000107-12-0	12.726	0.01
6	Catechol, 2TBDMS derivative	115421-28-8	17.967	0.13
7	1,4-Dimethoxy-2,3-dimethylbenzene	000000-00-0	19.061	1.88
8	Hexasiloxane, tetradecamethyl	000000-00-0	21.241	0.14
9	Oxalic acid, 2TMS derivative	055570-80-4	24.094	0.18
10	Hexadecanoic acid, methyl ester	000112-39-0	25.589	4.59
11	2-Hexenedioic acid, bis(trimethylsilyl) ester	055494-10-5	26.647	0.67
12	Hexatriacontane	000630-06-8	27.316	2.32
13	9,12-Octadecadienoic acid, methyl ester	002462-85-3	28.313	15.01
14	methyl dihydromalvalate	000000-00-0	28.411	7.00
15	cis-13-Octadecenal	058594-45-9	28.453	0.57
16	Methyl stearate	000112-61-8	28.754	1.31
17	Tetracosamethyl-cyclododecasiloxane	018919-94-3	28.998	1.84
18	2,3,4-trimethyl-1-pentanol	000000-00-0	31.011	1.04
19	Hexadecanoic acid	000057-10-3	31.208	6.71
20	Methylenecyclooctane	003618-18-6	32.889	0.36
21	SILICATE ANION TETRAMER	000000-00-0	33.097	0.29
22	E,E-1,9,17-Docasatriene	000000-00-0	33.294	0.27
23	15-Tetracosenoic acid, methyl ester, (Z)-	002733-88-2	33.372	0.66
24	Z,Z-6,13-Octadecadien-1-ol acetate	000000-00-0	33.61	26.49
25	OCTADECA-9,12-DIEN-1-OL	001577-52-2	33.673	21.74
26	2-Nitrocyclododecanone	095338-32-2	33.932	1.89
27	Undec-10-ynoic acid	002777-65-3	36.096	1.14
28	Undecane, 5-methyl-	001632-70-8	37.408	0.47
29	2,5-Dimethyl-4-methoxyphenol	000000-00-0	37.906	0.32
30	Methyl linolelaidate	002566-97-4	39.214	1.48

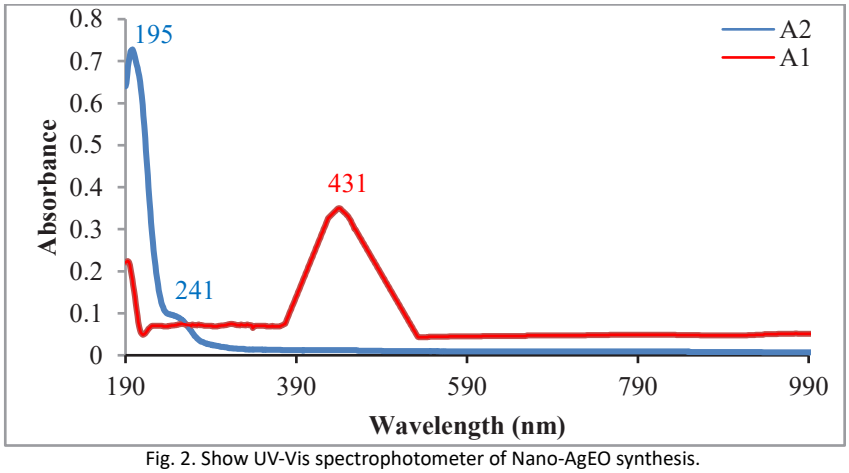

Fig. 2. Show UV-Vis spectrophotometer of Nano-AgEO synthesis.

aqueous extract of Nigella sativa L. seeds shown in Table 1 and Fig. 1 similar to Gerige which found the essential oils of Nigella sativa seeds contain 31 compounds [7]. Following a preliminary phytochemical examination, anthroquinones, alkaloids, amino acids, flavonoids, protein, steroids, and terpenoids were found. Plant extract was subjected to GC-MS analysis using

an Agilent Co. GC 7890A equipment, with mass spectrum interpretation. The database of the National Institute of Standards and Technology (NIST) was used for GC-MS. A total of twenty-eight compounds were identified by their peak values, retention time, and molecular mass. the major phytoconstituents such as Z,Z-6,13-Octadecadien-1-ol acetate (26.49%), Octadeca-9,12-dien-1-ol (21.74%), and 9,12-Octadecadienoic acid methyl ester (15.01%) were identified by high peak values similar to (8) which found 9,12-Octadecadienoic acid (z,z)- (10.39%) 9,12-Octadecadienoic acid (19.11%).

Characterization of Silver Nanoparticles

UV-Vis spectrophotometer

UV–Visible spectrum was recorded in the range for silver was 190–990 nm as previously reported (9). For AgNPs peak point was at 431 nm, that was analogous to the results found in previous studies of AgNPs as show in Fig. 2 [10].

Infrared Spectrometer (IR)

The FT-IR analysis was performed to find out the functional groups that were present in the extract and involved in formation of AgNP within the scanned wavelength range 400–4000 cm^{-1} Fig.

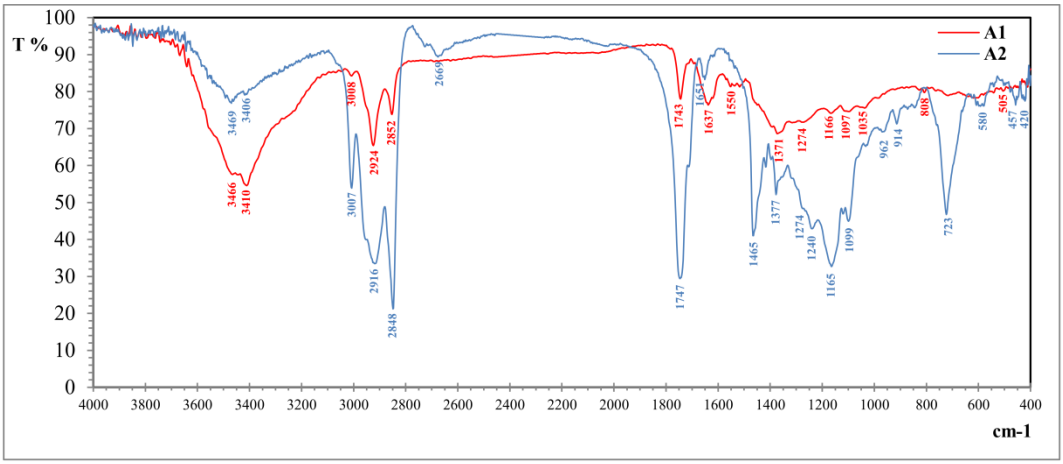

Fig. 3. Show IR Test of Nano-AgEO synthesis.

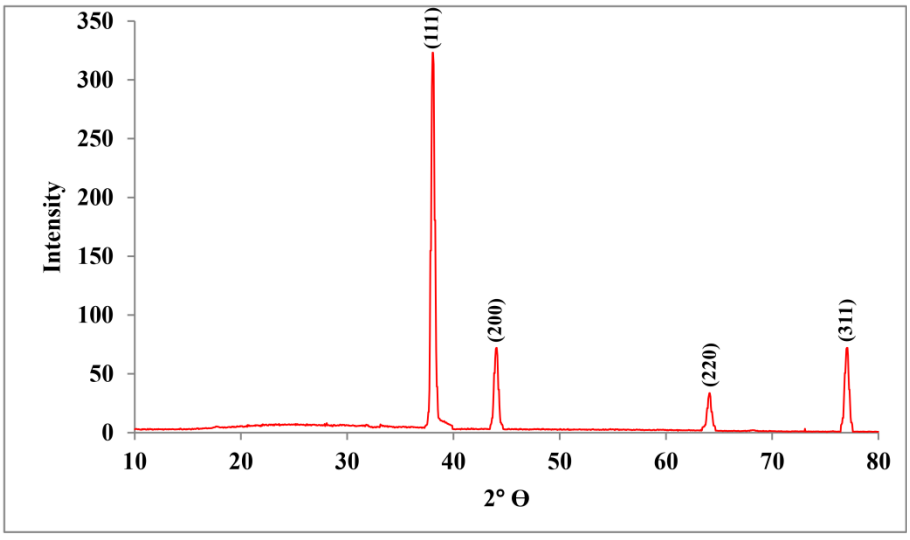

Fig. 4. Show XRD Test of Nano-AgEO synthesis.

3 It was found that the packets responsible for the bond O-H 4369 cm^{-1} had been exerted towards a lower wave number 4366 cm^{-1} when the formation of nanoparticles as well as the case in the C-H Aromatic Packs, where it showed a displacement from 3008 to 3007 cm^{-1} and the C-H Alphatic bond where it showed a displacement from 2916 and 2848 cm^{-1} to 2924 and 2852 cm^{-1} And the C=O bond where it showed a displacement from 1747 to 1743 cm^{-1} all these changes at the bonds site above, other than the changes that were not mentioned indicating the formation of AgEO [11–13].

X-ray powder diffractometer (XRD)

The XRD analysis was performed to confirm the crystalline structure of N. sativa AgNP which were confirmed by four distinct diffraction peaks that corresponds to 38°, 44°, 64°, 77° 2-Theta values, that were indexed to the (111), (200), (220) and (311) planes of silver Fig. 4. These results of XRD spectrum were authenticated by JCPDS file no. 00-001-1167 while similar results were also reported other researchers previously [11,14].

Field Emission Scanning Electron Microscopy (FESEM)

The morphology, shape, and size of the particles were characterized using FESEM (INSPECT 50, Spain), as shown in Fig. 5. The average size of AgEO was 35.9 ± 18.1 nm, with some particles being spherical and smooth, while most particles were irregular in shape. Based on these results, all formulations created showed spherical particles with a nanometer size of less than 100 nm [15].

Zeta potentials

The total charge of the particles in a medium is called the zeta potential. This is the potential energy at the limit of hydrodynamic shear. A

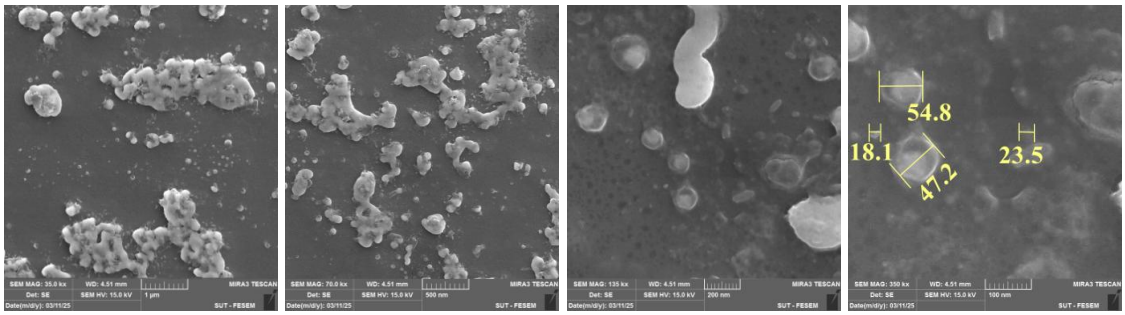

Fig. 5. Show FESEM Test of Nano-AgEO synthesis.

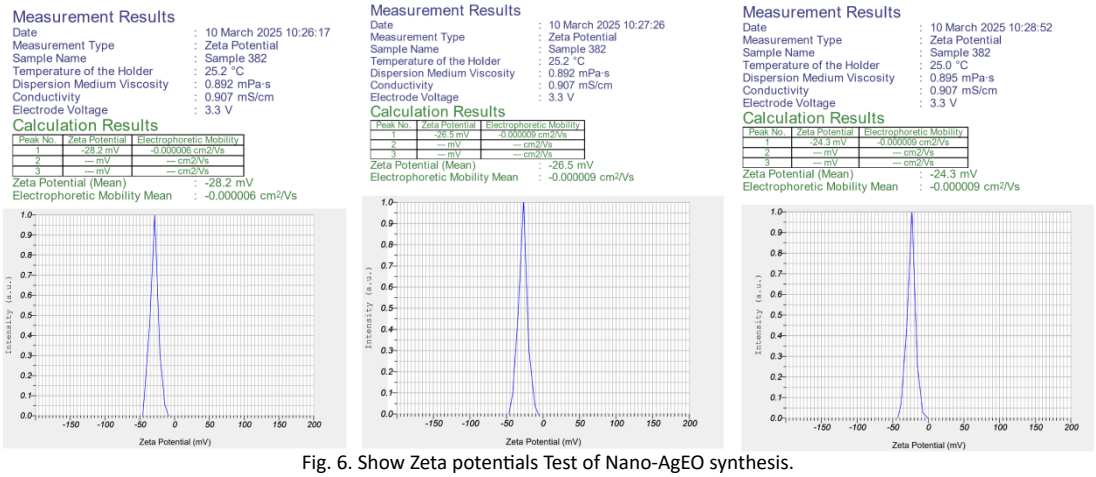

Fig. 6. Show Zeta potentials Test of Nano-AgEO synthesis.

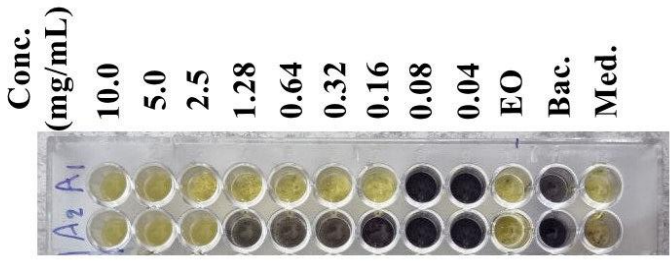

Fig. 7. MIC test against *Enterococcus faecalis*.

higher zeta potential indicates a more stable dispersion because all particles in the suspension tend to resist each other, avoiding aggregation. Stable particle suspensions typically have a zeta potential > +30 mV or < -30 mV. The stability, circulation time, protein interactions, particle cell permeability, and biocompatibility of essential oils can be determined by measuring the zeta potential. Zeta potential can improve the bioperformance of drug delivery systems by avoiding surface charge-related toxicity [16].

The results in Fig. 6 show that AgEO is -26.3 ± 2.8 mV. All AgEO formulations are considered to have high zeta potentials, which prevents them from aggregating or precipitating and gives them good stability. A previous study [17] calculated zeta potential values between -24.5 ± 0.9 and 19.7 ± 0.6 mV. Since -24.5 is close to -30 mV. High zeta potential and uniform particle size contribute to its good stability [18]. Another study by Risaliti [19] found a Z potential of -30 mV, which indicates that the system is very stable due to the strength of anionic charge repulsion to its surface.

In spite of the fact that, our comes about came inside the worthy ranges that are associated to the AgEO solidness and the variety in the comes about of zeta potential is due to a few components that influence the surface charge of the particles in colloidal frameworks such as concentration of phospholipid, sort and concentration of dynamic compound, ionic quality of the environment, and temperature.

Antibacterial Study

Determination of Minimum Inhibitory Concentration (MIC) and MBC of nano-AgEO's

The MIC results of nano-AgEO (A1) showed that the bacteria inhibitory concentration of 0.16 mg/mL, where the only essential oils (A2) show MIC in the concentration 2.5 mg/mL. MBC of nano-AgEO (A1) showed that the bacteria inhibitory concentration of 0.16 mg/mL, as shown in below Fig. 7.

Essential oil combinations with synergistic effects against various bacteria have been included by researchers. According to a study that supports the research, the synergistic process typically entails the inhibition of protective enzymes, a shared metabolic pathway, and the use of cell wall active substances that improve the absorption of other antimicrobial agents [20]. According to [21] essential oils have the ability to dissolve anionic lipopolysaccharides, permeabilize membranes, and increase bacterial cell sensitivity.

Enterococcus faecalis lacks a phospholipidic membrane in its cell envelope since it is a Gram-positive bacterium. When this barrier is absent in Gram-positive bacteria, the hydrophobic components can come into direct contact with the phospholipid bilayer of the cell membrane, where they can have an impact that either impairs the bacterial enzyme systems or increases ion permeability, allowing essential intracellular components to leak out [22].

The aforementioned results, which were consistent with those of a prior investigation, showed that Ns-AgNps had outstanding antibacterial activity [23]. In addition to killing various kinds of infections, silver nanoparticles have a significant bactericidal and bacteriostatic effect. In comparison to chlorhexidine, silver nanoparticles had a statistically significant bactericidal activity against S. mutans, according to Sadeghi et al.'s evaluation of the two substances' bactericidal effects [24].

CONCLUSION

The study offers yet another illustration of how Nigella sativa seed extract can be used to easily, cheaply, and non-toxically create nanoparticles

without the need of hazardous chemical reduction agents. Significant levels of activity, including an antibacterial impact and the suppression of biofilm formation against Enterococcus faecalis, were demonstrated by the biologically produced particles. In conclusion, our findings point to an economical and environmentally responsible method for the synthesis of AgEO, which could help develop new antibiofilm agents and treat bacterial infections that are resistant to several drugs.

The results of experiments showed that small particles had a better bactericidal proficiency than large silver-based NPs, supporting the conclusion that small silver nanoparticles (NPs) benefited from a higher surface area ratio. This study also suggests that essential oils can be used in place of chemical antibiotics due to their high effectiveness in stopping bacterial growth. The outer membrane of bacterial cells is disrupted by silver-based nanoparticles. Silver ions may also interfere with bacterial DNA to stop the reproductive system of the organism from working.

CONFLICT OF INTEREST

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

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