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*Research Article*

# Preparation of Nano Magnesium Oxide Loaded with Syrian Inula Plant Extract And Study of antibacterial activity Against *Vibrio cholera.*

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ABSTRACT

**Background:** Nanotechnology is considered one of the best advanced technologies in treatment through its ability to transport effective drugs and compounds and release them more effectively. MgO nanoparticles showed antimicrobial properties against both Gram-negative and Gram-positive bacteria in vitro, including Escherichia coli and Staphylococcus aureus, as *Vibrio cholera* bacteria was selected during the period when the epidemic began to appear in Syria, with the presence of several people showing symptoms of diarrhea in the city of Aleppo.

**Methods:** In this research, Nano magnesium oxide particles were prepared by sol-gel method, then the active compounds were extracted from the Syrian Inula plant using ethanol as a solvent. next step was load the plant extract on the surface of the prepared magnesium oxide particles. The antibacterial activity of the new product was studied against the bacteria that cause cholera.

**Results:** Its effectiveness was compared with the effectiveness of 7 different antibiotics, and it outperformed all of them, noting that the studied isolate was resistant to three of them.

**Conclusion:** The combination of nanoparticles with the natural extract is more effective in fighting bacteria, because each of them works in a different way and affects differently, so the combination of the two technologies gives greater effectiveness.

## Introduction

Cholera is a twisted gram-negative bacillus varying in size from 1 to 3 µm in length by 0.5 to 0.8µm in periphery. It belongs to the family Vibrionaceae and shares common characteristics with the family Enterobacteriaceae. The bacterium has a single polar flagellum that confers the erratic movement on microscopy (1,2).

The *Vibrio cholera* is responsible for roughly 120 000 deaths every time and has a major impact on the health of youthful children between the periods of 1 and 5 times (3).

Cholera is contracted by ingestion of food or water defiled with the Gramnegative bacterium *Vibrio cholerae*. The bacteria pass through the mortal gastric acid hedge into the small intestine where they populate, multiply and begin to cache cholera poison. Because this organism is sensitive to the low pH set up in the mortal stomach, a high contagious cure of 108 bacteria is needed for the onset of severe cholera; still, the contagious cure can drop to 104 bacteria in individualities who produce lower stomach acid, including youthful children, the senior and those who take antacids (4).

About 1–5 days after ingestion, cholera cases witness unforeseen watery diarrhoea and vomiting. Up to 20 liters of watery diarrhoea, appertained to as ricewater coprolite, are exfoliate diurnal containing 109 *Vibrio cholera* pe millilitre of coprolite (5).

Water loss caused by cholera may reach one liter per hour in grown-ups, leading to severe dehumidification, shock and eventual death. still, the cholera casualty rate reaches 50 within a many hours to days after onset of the complaint (6).

The environmental continuity of *Vibrio cholera* in the submarine terrain can be attributed to multiple intra- and interspecific strategies similar as responsive gene regulation and biofilm conformation on biotic and abiotic shells, as well as relations with a multitude of other organisms. Cholera remains a patient cause of morbidity and mortality in Asia and Africa. In 1998, the World Health Organization (WHO) entered reports of 293121 cholera cases and 10586 deaths from 74 countries, with the factual number of cases (including unreported cases) presumably greatly exceeding this figure. Cholera can also move strongly through populations that have been free of the complaint for generations, as demonstrated by the circumstance of over 100000 cases in Peru in 1991 and the posterior rapid-fire spread of cholera to other South American countries (7).

We chose this plant because of the lack of studies conducted on it in Syria on the one hand, and because the results of the chemical analysis, according to many reference studies, confirmed that it contains flavonoids, tannins, and triterpenoids, which have antibacterial effectiveness

## Materials and Method

### Preparation of magnesium oxide nanoparticles

Magnesium oxide nanoparticles were synthesized using agnesium nitrate ($Mg(NO_3)_2.6H_2O$) as asource material with sodium hydroxide. For the typical experimental procedure; 0.2M magnesium nitrate ($Mg(NO_3)_2.6H_2O$) was dissolved in 100 ml of deionized water. 0.5M sodium hydroxide solution was added drop wise to the prepared magnesium nitrate ($Mg(NO_3)_2.6H_2O$) solution while stirring it continuously. White precipitate of magnesium hydroxide appeared in beaker after few minutes. The stirring was continued for 30 minutes; pH of the solutions was 12.
precipitate was filtered and washed with methanol three to four times to remove ionic impurities and then centrifuged for 5 minutes at 5000 rpm/min and dried at room temperature. The dried white powder samples were annealed in air for two hours at 500°C.

### Isolation of the active components in the Inula plant

The Inula plant was collected from northern Lattakia in Syria, where the plant was completely dried (the stem with leaves and flowers) for four days at laboratory temperature and away from sunlight. grams of dry powder of the ground plant in 150 ml of ethyl alcohol for a day, then put the mixture in an ultrasound machine for 30 minutes at a temperature of 50oC, the ethanolic extract was concentrated using a rotary evaporator, then dried using an electric oven at 40◦C to obtain Pale green powder estimated at 4.72% of dry weight of the plant.

The chemical components of the plant extract were determined by GC-MS using an HP-5MS 5% Phenyl Methyl Silox column with dimensions of 30m x 250µm x 0.25µm, the carrier gas being helium, a flow velocity of 0.9 mL/min, injector temperature 250oC and detector 280oC. Using the reference spectra in the GC-MS library.

### Inoculation of prepared nano- magnesium oxide with plant extract

plant contains many compounds with antibacterial activity that have been studied individually in previous research. The aim was to benefit from the entire plant extract with all its components because each compound is characterized by a different specific effectiveness from the other, and isolating the compound from others requires multiple stages, so Using the whole extract allows higher utilization in simpler stages. 10gr of the prepared nano magnesium oxide was taken and added 30ml of absolute ethyl alcohol, the mixture was subjected to ultrasonic waves with stirring to obtain a good homogeneity of the components, in parallel with the above, an extract of 5gr of Inula plant was prepared as shown in this research without daring. Ethanolic extract of the plant was added to the magnesium oxide solution gradually with rapid stirring, the stirring was continued with heating to 50◦C for 30min. The mixture was dried in an electric oven at 70◦C until a light green dry powder was obtained representing nano-hydroxyapatite with Inula extract, where the ratio (magnesium oxide: Inula) was equivalent to (1:0.5). product was stored in a glass container isolated from light and moisture for subsequent tests.

### Identification and characterization of the bacterial isolates

The bacterial isolate were obtained from a sample of diarrhea of a person showing symptoms of cholera infection.

it was plated onto Thiosulfate Citrate Bile Salt Agar (TCBS) and Yellow colonies from the plate were randomly picked and were further confirmed by biochemical tests as described in Bergey's Manual of Systematic Bacteriology They were Gram-negative, motile bacilli, and catalase-oxidasepositive. Growth occurred at 37°C and at 0.0–6.0% (w/v) NaCl not above, production of indole and acetoin (Voges–Proskauer), fermentation of glucose (no gas), maltose, mannitol and sucrose and nitrate reduction to nitrite; they do not utilize arabinose, and sorbitol.

Sensitivity of the studied isolate was tested for 7 antibiotics, namely, Tetracyclines, Ampicillin, Gentamicin, Chloramphenicol, Colistin, Polymyxin B, and Imipenem Table 1, according to laboratory's antibiotic discs. Results were interpreted using CLSI (Clinical and Laboratory) standards. (8).

**Table 1.** antibiotics used to study isolated Sensitivity

| | Antibiotic | Antibiotic Code |
|---|---|---|
| 1 | Tetracyclines | TE |
| 2 | Gentamicin | GEN |
| 3 | Chloramphenicol | C |
| 4 | Ampicillin | AMP |
| 5 | Colistin | COL |
| 6 | Polymyxin B | PB |
| 7 | Nalidixic acid | NAL |

The antibacterial activity was studied by agar diffusion method, the bacteria were planted on Mueller-Hinton solid medium with a $1.5*10^{6}$ CFU. The chemical compound was added in different quantities (25-50-75-75-100)µL The dishes were incubated at 30°C for 24 hours.The diameters of the zone inhibition were measured and the average was taken.

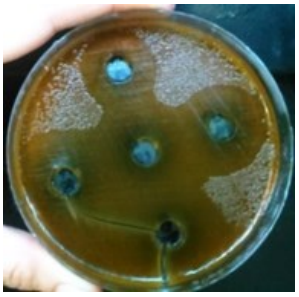
## Results

The most important chemical components of the studied plant were identified as shown in Table 2.

**Table 2.** The most important chemical components of the studied Inula

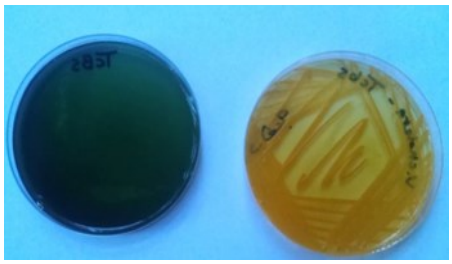
| compound | % |
|---|---|
| Linalyl propionate | 18.21 |
| Eugenol | 16.32 |
| Benzophenone | 7.88 |
| β-Linalool | 6.21 |
| Palmitic acid | 4.12 |
| n-Heptadecane | 3.87 |
| Edulan I, dihydro- | 3.76 |
| Himachalol | 3.43 |
| Linoleic acid | 3.11 |
| p-Cymen-8-ol | 2.96 |
| α-Copaen-11-ol | 2.67 |
| Caryophyllene oxide | 1.98 |
| n-Tetradecane | 1.89 |
| α-Costol | 1.43 |
| n-Octadecane | 0.76 |

Antibacterial activity of nano Magnesium oxide Loaded with Syrian Inula in Figure 1



Antibacterial activity of the chemical compound (mm)

Sensitivity of *Vibrio cholera* to antibiotics



*Vibrio cholera* on TCBS agar

**Figure 1.** antibacterial activity of nano Magnesium oxide Loaded with Syrian Inula

## Discussion

according to Bergey's Manual of Systematic Bacteriology 2005 the type of bacteria was belong to *Vibrio cholera*.

The bacterial isolate was recorded as susceptible to Tetracyclines, Gentamicin, Chloramphenicol, and Nalidixic acid and risistance to Ampicillin, Colistin , and Polymyxin B according to clsi (clinical and laboratory) standards. (8) while The prepared new material showed high antibacterial activity against *Vibrio cholera*, and the diameters of the zone inhibition ranged between 25 mm and 34 mm. When comparing the effectiveness of the new prepared substance with the effectiveness of antibiotics , its effectiveness exceeded the effectiveness of all studied antibiotics Figure1.

The combination of nanoparticles with the natural extract is more effective in fighting bacteria, because each of them works in a different way and affects differently, so the combination of the two technologies gives greater effectiveness.

## Conclusion

Magnesium oxide nanoparticles prepared by sol-gel method and loaded with the extract of the active compounds present in the Syrian Inula plant, were effective against cholera-causing bacteria. Its effectiveness was compared with the effectiveness of 7 different antibiotics, and it outperformed all antibiotics, noting that the studied isolate was resistant to three of them.

### Competing interests

The authors declare that they do not have any conflicting interests.

### Data and materials availability

All data are available in the main text or the supplementary materials.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Aleppo university


### Authors contributions

Dr.Abdulrazzaq Hammal: Chemical section.
Dr.Hiba Al-Hamed Al-Duihi: Microbiological section.


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