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# Antibacterial Activity of Chitosan Nanoparticles With *Ocimum basilicum* oil Extract on *Pseudomonas aeruginosa*

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**Abstract:** The present study aims to describe the antibacterial activity of chitosan nanoparticles (CSNPs ) loaded with Essential Oil of *Ocimum basilicum* (BEO) extract against Multiple drug resistance MDR *Pseudomonas aeruginosa*. The aim of the study effect antibacterial activity of chitosan nanoparticles (CSNPs ) loaded with Essential Oil of *Ocimum basilicum* (BEO) extract against Multiple drug resistance MDR *Pseudomonas aeruginosa*. The 40 isolates of *Pseudomonas aeruginosa* were collected from patients suffering from burn infection.The isolates were diagnosed as *P. aeruginosa* by classical biochemical tests and PCR technique using 16S rRNA gene as a diagnostic gene and gel electrophoresis. The sensitivity of bacteria to antibiotics was tested using the Kerby-Bauer method. The antibacterial activity for This was determined by using the Agar well Diffusion Method (ADM) for Essential Oil of Ocimum basilicum and the Disc Diffusion Method for chitosan nanoparticles loaded Essential Oil of Ocimum basilicum.The results showed that both the test substances have antibacterial activity against *Pseudomonas aeruginosa* isolates with superior inhibition of nanoparticles at low concentrations. The current study concluded the possibility of using chitosan nanoparticles loaded with Essential Oil of Ocimum basilicum extract as a natural alternative against MDR *Pseudomonas aeruginosa* due to the nanoparticles' unique properties that increase drug effectiveness and efficiency in drug delivery.

**Keywords**: Chitosan nanoparticles, *Pseudomonas aeruginosa*, *Ocimum basilicum* , Essential Oil , MDR.

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## Introduction

Plants produce a variety of chemicals for biological purposes, such as repelling insects, fungi, and herbivores. Science knows about 12,000 active ingredients. The effect of these substances on the body is exactly the same as prescription drugs. The World Health Organization has reported (1) that at least 75-95% of people worldwide still use plant extracts or of their essential oils to treat diseases. Today the world is questioning essential oils and plant extracts for their potential as a source of physiologically active substances and free radical scavengers. *Ocimum bacilicum*, or sweet basil in English, belongs to the Lamiaceae family. The basil plant has remarkable therapeutic properties, particularly in its fragrant leaves. It has long been used in traditional medicine in the treatment of upper respiratory tract infections, as well as tonic, antibacterial, diuretic and antispasmodic.

*Pseudomonas aeruginosa* is an opportunistic pathogen with a high level of treatment resistance. because of the outer permeability barrier which it is naturally resistant to many antibiotics (2). Chitosan is a naturally occurring polycationic deacetylated polymer composed of D- and N-acetylglucosamine groups. Chitosan is available in different molecular weights, has fascinating properties and can be used in various processes, including medicine and Industry. Nanoparticles (NPs) are small substances (1–100 nm) that can function as complete entities. These chemicals can be used as antimicrobial therapeutic agents, as well as in consumer products. Bionanotechnology is an exciting and evolving engineering technique for synthesizing reliable and environmentally friendly nanomaterials from biological sources Pharmaceutical side effects can be reduced by using green nanoparticles for drug delivery (3).

## Materials and methods

### Samples collection

The 40 isolates of *Pseudomonas aeruginosa* were collected from burn patients in hospitals in Baghdad. Specimens were collected using sterile swabs and transport medium and cultured on blood agar medium, MacConkey agar medium and cetrimide agar (for bacterial isolation and diagnosis, incubated for 24 hours at 37°C for further testing).

### Biochemical tests

The isolates underwent a standard biochemical assays to verify isolates their identity, these included the following: the Gram stain, Catalase production, the Oxidase test, the formation of Indole and Urease, the fermentation of Lactose at 42 °C, and the microscopic shape. All tests were carried out in accordance (4).

### Genotypic identification

Bacterial DNA was extracted from all isolates using Genomic DNA extraction kit, purification depends on the protocol of manufacturing company (Geneaid, Taiwan). with Active before Starting DNA Extraction by culture was inoculated in 10 ml of Brain heart infusion broth medium and was incubat ed over night at 37°C.

### Identification gene selection

In this study, a traditional polymerase chain reaction (PCR) was used to identify the 16S rRNA gene which can be used to identify *P. aeruginosa*. 16srRNA primer (188bp) was, provided.by (Macrogen, Korea) F (5'GAGAGAGGGCAACTCGCTAC-3") and R (5'ACAACCTGCTGGAC TATCGC-3’). PCR products were visualized on a 1% agarose gel stained with a red safe dye (5).

### Preparation of primers

The primers were used to detect the gene using a standard PCR experiment. Lyophilized forward and reverse primers (purification depends on manufacturer's protocol) were converted to a stock solution with a final concentration of 100 pmol/µl when dissolved in nuclease-free water. To prepare a convenient 10 pmol/µL primer solution, 10 µL of Primer Stock Solution (stored in a -20 °C freezer) was mixed with 90 µL of nuclease-free water.

### Amplification reaction

The PCR mixture reactions were carried out in a total volume of 20 µl. 2 µl DNA template amplified using 10 µl of Taq PCR PreMix and 1 µl of each primerTo achieve the final volume of 20 µl, the mixture reaction was mixed and centrifuged for 3 seconds to collect wall droplets. The mixture

reaction was then transferred to a thermal cycler to begin the reaction in accordance with the instructions of the particular program.

**The cycling conditions of the *16S rRNA* gene**

The PCR amplification program used in the current study consisted of the following steps: initial denaturation at 95 ℃ for 5 minutes, denaturation at 95 ℃ for 45 seconds, annealing at 56 ℃ for 45 seconds, extension at 72 ℃ for 1 minute, repetition for 35 cycles, and final extension at 72 ℃ for 5 minutes 1cycle.

**PCR product electrophoresis on agarose gel**

The DNA was detected after extracting it as follows : Aliquot of 100 ml of 1X TBE were taken in a flask. Amount of 1.5 gram (for 1.5%) agarose is added to the buffer. Aliquot of 4 µl of Red Safe dye is added to the agarose solution. And then, the agarose stirre in order to get mixed The extracted DNA sample of *Pseudomonas aeruginosa* isolates and the ladder marker were resolved by electrophorysis. Three µl of louding Dye plus 6 µl of extracted DNA samples was loded on agarose gel and run of for 90 min at 5V/cm. Finally, the band was visulized on UV transiluminater and then photograophed by using a gel documentation system (6).

**Antibiotic susceptibility assays**

The antibiotics chosen were Piperacillin-tazobactam, Bacitracin, Aztreonam, Gentamicin, Imipenem, Colistin, Amikacin, Piperacillin, Ciprofoxacin, and Ceftazidime, according to the Institute of Clinical Standards and Laboratories' (7) guidelines. The Kirby-Bauer technique, as described by (8), was used to prepare antibacterial susceptibility tests. Briefly, after an overnight nutrient agar plate culture, 1-2 colonies were transferred to 3 mL of normal saline.A 1.5×108 CFU/ml) McFarland adjustment was made to the turbidity. Different antibiotic discs were employed, placed on the surface of the medium with sterilized forceps, and then the plates were inverted and incubated at 37°C for 18–24 hours. The inoculation procedure involved using a sterile cotton swab dipped in the bacterial solution to inoculate Muller Hinton agar plates. The zones of inhibition that resulted were quantified and compared with (7) breakpoints. According to (9), the isolate's sensitivity, intermediate status, or resistance to a particular antibiotic was determined by comparing it to traditional inhibition zones.

**Extraction of the essential oil of basil**

Distillation is used to isolate essential oils. According to the literature, dried basil plants are weighed (100 g) and thenplaced in a distillation apparatus filled with water up to the filter boundary (0.5 L distilled water) and subjected to hydro distillation for four hours using Clevenger device apparatus (10).

**Biosynthesis of chitosan/oil of *Ocimum basilicum* Nanoparticles**

The chitosan molecules were loaded into the essential oil in two steps: oil-in-water emulsification and ionic gelation. Chitosan amide was separated by filtering, washed multiple times with methanol, hot distilled water, and ethanol, dried in an electric oven at 50°C, and weighed in the presence of xylene using the Dean-Stark (Clevenger) apparatus and an equal volume of Chitosan 5% (200 mg of CS +100 ml acetic acid).Chitosan NPs loaded BEO (CSNPs loaded BEO) was made by mixing Tripolyphosphate (TPP) with the CS- BEO adduct as follows: TPP was added to the CS-BEO

solution in a 1:2.5 (w/w%) ratio with continuous stirring at room temperature for 6 hours after dissolving 5 mg/ml CS-BEO adduct in acetic acid solution (1% w/v). An ionic-gelation approach was used to create the CSNPs loaded BEO. The nanoparticles were separated, washed multiple times, and the liquid layer was removed before the precipitate was resuspended in water and dried (11).

**Characterization of chitosan nanoparticles loaded essential oil of ocimum basilicum**

In this investigation, several different methodologies were applied to describe (morphological and structural) and detect CSNPs loaded BEO.

**Antibacterial activity of test materials**

A test was carried out on ten isolates out of forty of *Pseudomonas aeruginosa*, which are highly resistant to antibiotics.

**Antimicrobial activity of essential oil of *Ocimum basilicum* by agar well-diffusion method (ADM)**

The same procedure for antibiotic sensitivity test was followed:

Wells were made into the agar by a cork borer, 100 µl of BEO were transferred into the agar well (concentrations were 7%, 5%, 2.5%, 1.25%, and 0.625%), Plates were incubated for 24 hours at an incubation temperature of 37 °C in aerobic conditions and After the incubation period, the size of the inhibition zone around each well was measured.

**Antimicrobial activity of the BEO-loaded chitosan nanoparticles by disc diffusion method**

The disc diffusion technique for antimicrobial susceptibility testing was used. . The discs (6mm in diameter) were put on the Mueller Hinton agar surface at identical distances after being impregnated with a variety of concentrations of BEO-loaded chitosan nanoparticles (100, 50, 25, 12.5, and 6.25%). The plate was then incubated for 24 hours at 37°C. The plates were tested after incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using calipers and recorded. The test were repeated three times to ensure reliability (12).

**Results and discussion**

**culturing examination**

The forty isolates were tested primarily to characterize the colonies by examining the phenotypic properties of the bacteria by growing them on MacConkey agar medium, as some colonies exhibited a pale due to their inability to ferment the sugar lactose (13). As for the blood agar medium, some colonies showed the ability to partially degrade blood by β-hemolysis, testifying to the ability of the bacterium to produce the enzyme-hemolysin (14). Agar medium with cetrimide as is. Some colonies are greenish-yellow and called pyoverdin, or greenish-blue called pyocyanin, which fluoresce when exposed to UV-UV light (15).

Microscopy was performed because examination revealed the presence of nonsporulating gram-negative rods. Biochemical tests were performed and the isolates showed positive results in every oxidase and catalase test. In the biochemical tests, which included the indole test and the methyl red test, the results were negative and . Results in the urease assay were inconsistent, showing their ability to grow at 42°C. (Table 1) shows the results of these studies.

**Genotypic identification of *P.aeruginosa* isolates**

The results demonstrated the presence of the 16s rDNA gene in the

isolates included in the study and all were carriers of the gene (100%) as determined by polymerase chain reaction of the rRNA gene. Comparison of double beams with a size guide (DNA ladder) with particle beams of known size provided by Macrogen, Korea. It was noted that the sizes of the bundles are similar to the expected size When compared with the results reached by (16).As in (Figure 1). 16S rRNA-based PCR tests can distinguish *P. aeruginosa* from other phylogenetically similar Pseudomonas species quickly, easily, and reliably.

**Table (1): Morphological and Biochemical Identification Results of *P. aeruginosa*.**

| No. | Biochemical Test | Result |
|---|---|---|
| **1** | Gram stain | - |
| **2** | Catalase production | + |
| **3** | Oxidase test | + |
| **4** | Indole production | - |
| **5** | Urease production | +/- |
| **7** | Growth at 42°C | + |
| **8** | Microscopic shape | Rods |

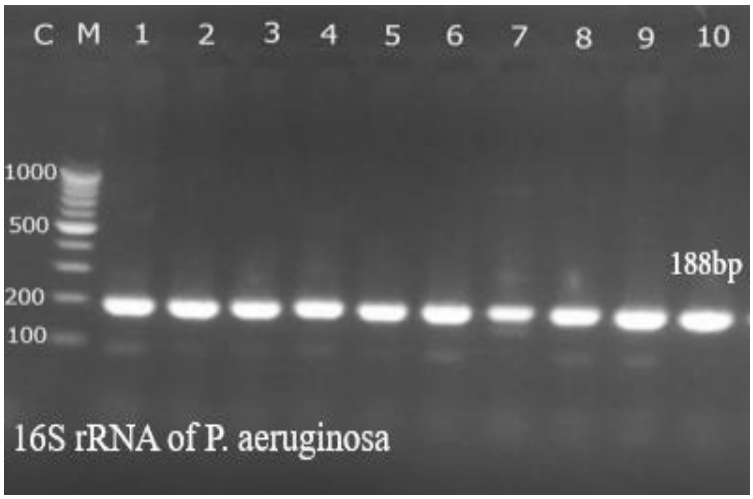
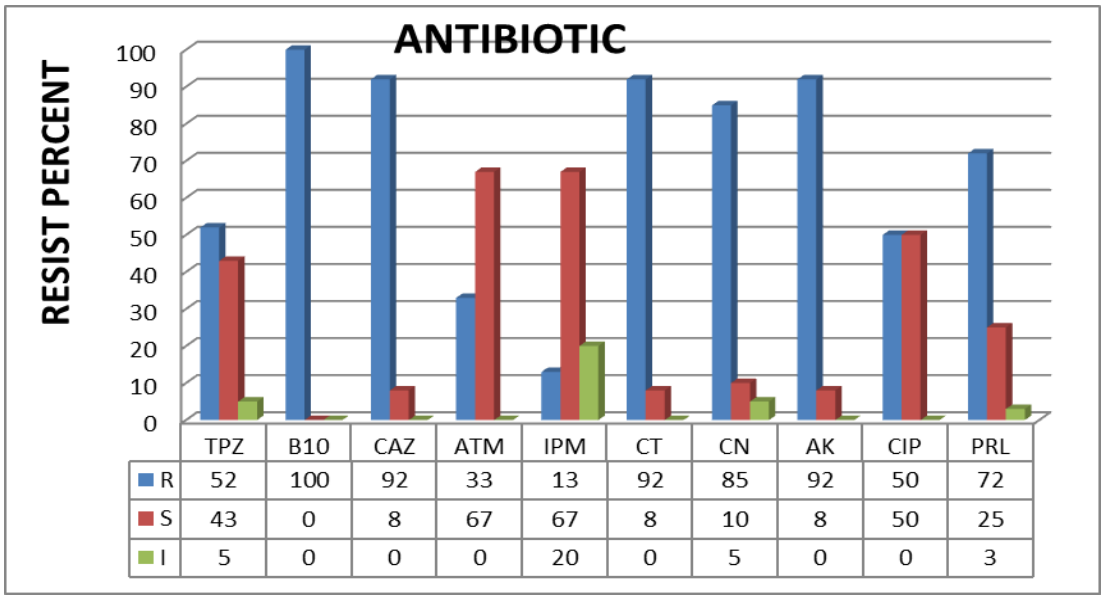



**Figure (1): Amplicons of 16S rRNA gene fragments after electrophoresis on agarose gel (1.5%) for 90 min at 5V/cm.**

## Antibiotic susceptibility assays

The antibiotic susceptibility of *P. aeruginosa* isolates was determined by the disk diffusion method (DDM) with the guidelines of CLSI, (7) depending on the diameter of the inhibition zone (mm). This assay was conducted on all 40 isolates against 10 antibiotics. The results revealed that most isolates have a very high level of resistance to the antibiotics used in this study, where the isolates vary in their susceptibility to the antibiotics.In the present study, most of the isolates were Multi-Drug Resistant (MDR). The result of the susceptibility test is presented in (Figure 2) which shows the resistance percent of *P. aeruginosa* isolates of the 40 *Pseudomonas aeruginosa* isolates tested, 40 (100%) isolates were bacitracin resistant, followed by 37 (92%) amikacin and ceftazidime and colistin resistant isolates, followed by 34 (85%) gentamicin resistant isolates of 29 isolates (72%). resistant to piperacillin,

while 8 isolates (20%) were moderately resistant to imipenem, 27 isolates (67%) were susceptible to aztreonam and imipenem, followed by 0.20. Isolates (50%) were resistant to ciprofloxain, 17 isolates (43%) were susceptible to piperacillin-tazobactam. One of the worrying characteristics of *Pseudomonas aeruginosa* is its low sensitivity to antibiotics, attributed to this ability, which is either normal or acquired through mutation of its genetic material or through horizontal gene transfer (17).




| | TPZ | B10 | CAZ | ATM | IPM | CT | CN | AK | CIP | PRL |
|---|---|---|---|---|---|---|---|---|---|---|
| R | 52 | 100 | 92 | 33 | 13 | 92 | 85 | 92 | 50 | 72 |
| S | 43 | 0 | 8 | 67 | 67 | 8 | 10 | 8 | 50 | 25 |
| I | 5 | 0 | 0 | 0 | 20 | 0 | 5 | 0 | 0 | 3 |

**Figure (2): Chart of Antibiotic Susceptibility assay for *P. aeruginosa* Using 10 Antibiotic agents.**

**Characterization of oil of *Ocimum basilicum* loaded chitosan NPs**

**Uv-vis spectroscopy**

Uv-visible spectroscopy is used to validate the synthesis of CSNPs-laden BEO. The absorbances of BEO and CSNPs-loaded BEO were tested; the results are shown in (Figure 3,4) for BEO and CSNPs-loaded BEO respectively.

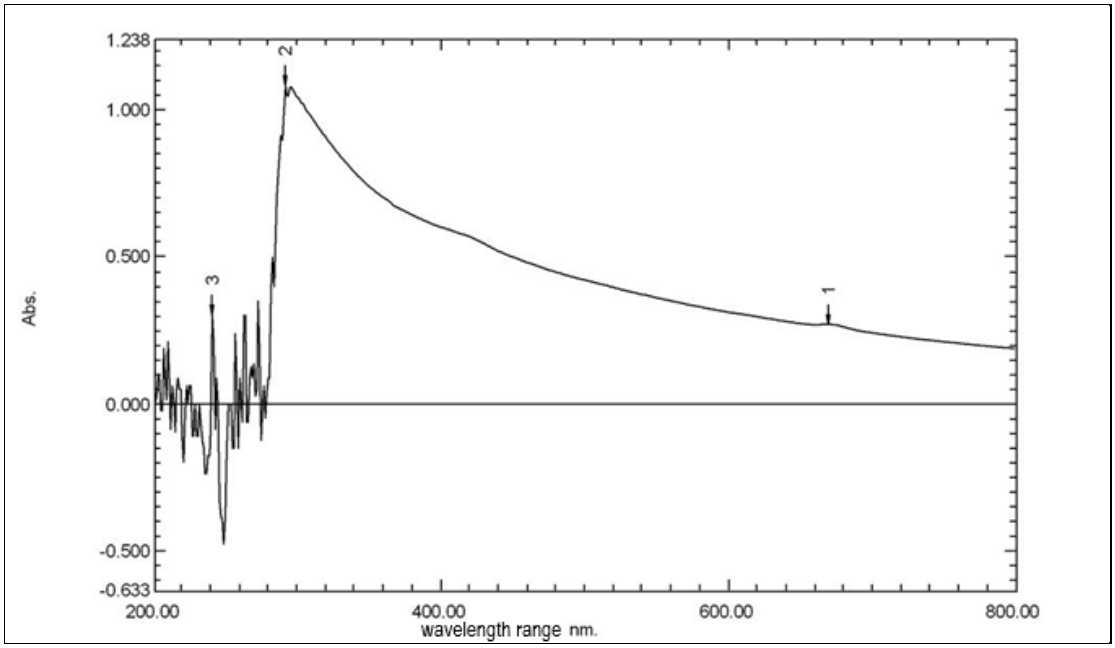



**Figure (3): UV-Visible spectral analysis of BEO**

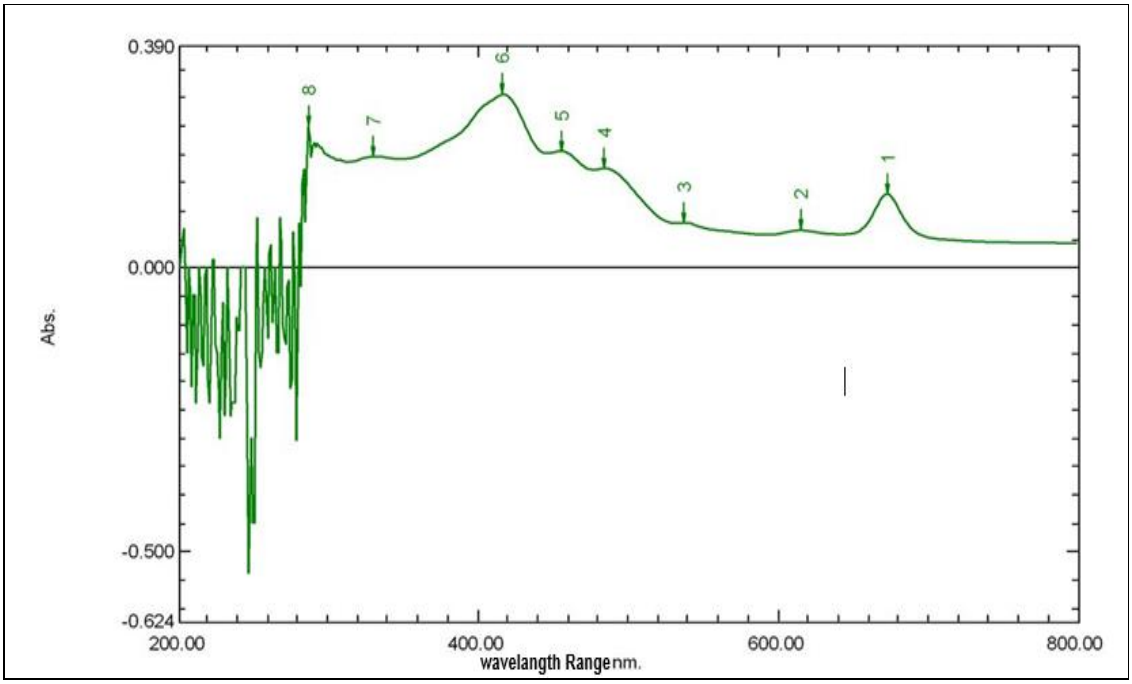



**Figure (4): UV-Visible spectral analysis of CSNPs loaded BEO.**

The greatest absorbance value in the BEO was 1.082, while the lowest absorbance value was 0.301 at wavelengths 240 nm and 291 nm, respectively. For CSNPs loaded BEO, the greatest absorbance value was 0.248 and the lowest absorbance value was 0.196 at wavelengths 286.00 nm and 329.00 nm, respectively. The decrease in absorbance value at wavelength 669.00 nm from 0.270 in BEO to 0.065 nm in CSNPs loaded BEO, as well as the highest absorbance value at wavelength 286.00 nm with a value of 0.248 in CSNPs loaded BEO material after it was the highest value in BEO at a wavelength of 291.00 nm with a value of 1.082, Along with the emergence of a

new wavelength with strong absorption, the highest of which was at 416.00nn in CSNPs with a value of 0.306 and all loaded with BEO, showing the successful formation of nanomaterial and loading of BEO on the chitosan nanoparticles.

**Fourier trans formation infrard spectroscopy (FTIR)**

In our study, we have confirmed functional groups of chitosan and presented converted chemical groups of chitosan-loaded plant essential oil. The result of our tested material was present in (Figure 5) shows the spectra of chitosan as it appears the existence of C-H stretch was shown by the peak at 2924.09 cm-1, while the peak at 3427.51 cm-1 indicated symmetric stretching vibration of O-H. Furthermore, C=O stretching caused the peak at 1523.76 cm-1, and amide stretching caused the peak at 1134.14 cm-1.Whereas (Figure 6) shows the spectra of CSNPs loaded BEO. The peak observed at FTIR spectrum has several peaks, the first at 447.49cm−1 which represents out-of-plan bending NH and out-of-plan bending C=O. The absorption peaks at 615.29 cm−1 are attributed to the vibration of –C≡CH. The absorption peaks at 943.19 cm−1 and 993.34 are attributed to the vibration of C-0-C . Peaks at 1149.57 cm−1 are related to in-plane O–H bending oaromatic compounds, this functional group is due to the presence of favones, and terpenoid compounds in the oil basil extract. When comparing the spectra of CS and CSNPs loaded BEO in the beginning, a new spectrum appeared at 447.49, 2185.35, and 2090.84 cm-1 that was not present in CS, which indicates the emergence of a new bond as a result of the emergence of a new compound. While the intensity of the main peaks was decreased for CSNPs loaded BEO than CS, that's due to the reduction of the intensity of hydrogen bonds via the cross-linking of TPP. This indicates that the large molecules were broken into smaller molecules and when CS has linked with TPP the value of the surface area of the bond increases and the area increases. Changes in the functional groups of active biomolecules indicate that they are related to the formation of CSNPs loaded BEO The unchanged structure and role of the chitosan-loaded essential oils indicated that the nanomaterial increased their biological properties (18).

**X-ray diffraction (XRD)**

The chitosan X-ray diffraction patterns indicate a major peak of 2θ value at 20.53° and an intensity level similar to 1200 cont. (Figure 7), on the other hand, depicts the XRD pattern of a BEO sample containing CSNPs. Five distinct peaks at 2θ were at 18.88, 26.67, 31.93, 35.72, and 38.04° (Figure 8), this change indicates the difference in the crystal structure between these two materials where CSNPs loaded BEO was more crystalline than CS showing a shift from the peaks normal chitosan. In this investigation, the CS diffraction peak, which was previously discovered at 20.53°, has migrated to a higher value of 38.04°. This might be linked to the interaction of CS loaded with BEO to generate CSNPs loaded BEO. According to (19), the apex lies approximately 2θ = 30°. It might be a symptom of the existence of some elements loaded on chitosan, which backs up the study's conclusions concerning the high degree of crystallization of chitosan loaded with basil essential oil.

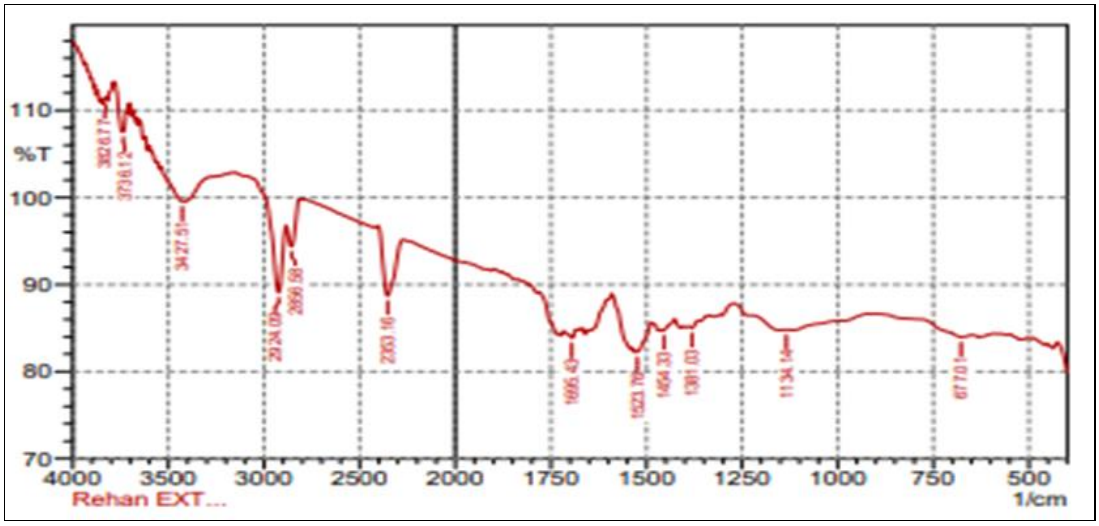



**Figure (5): FTIR Spectra Pattern of CSNPs loaded BEO**

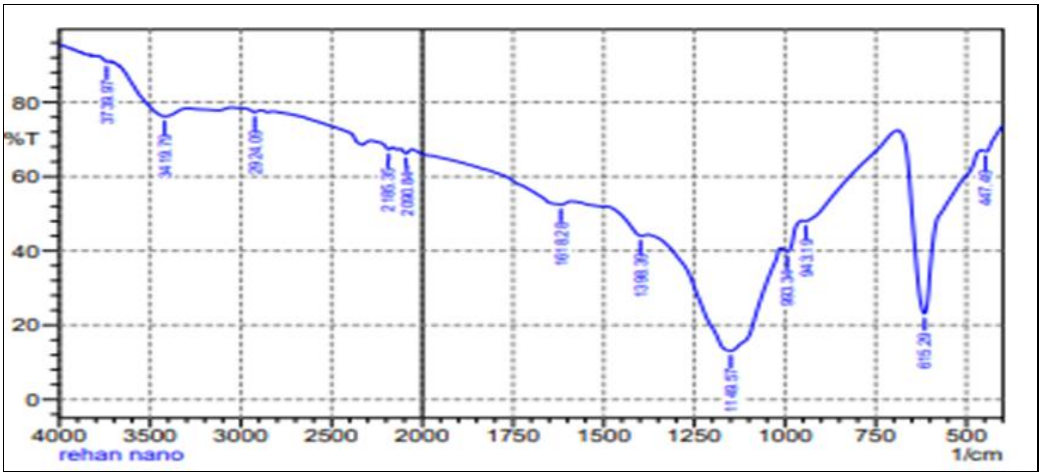



**Figure (6): FTIR Spectra Pattern of CSNPs loaded BEO**

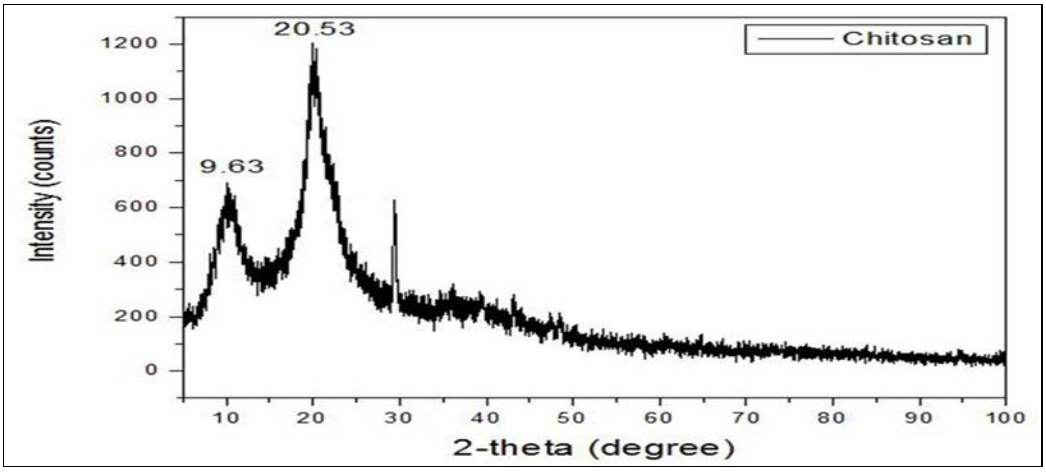



**Figure (7): Diffractogram of CS**

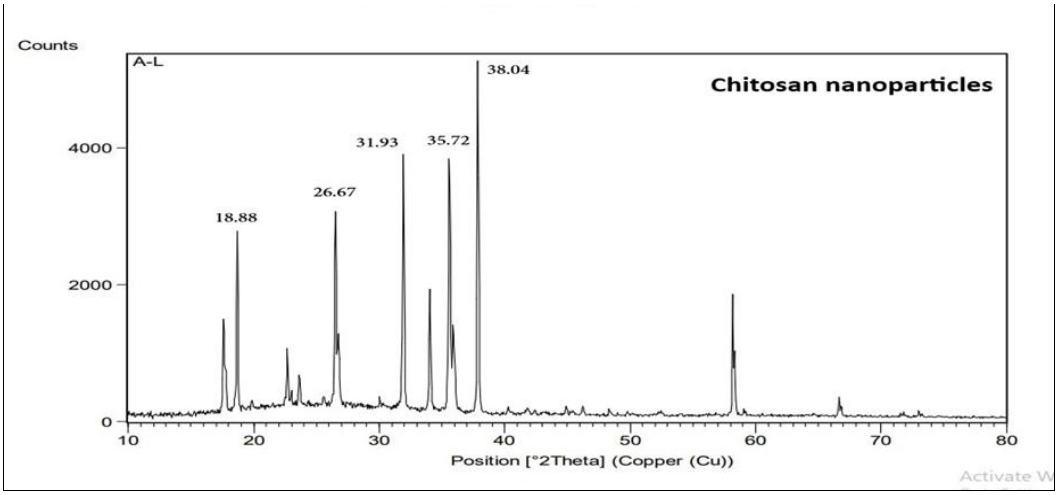



**Figure (8): Diffractogram of CSNPs loaded BEO**

## Antibacterial Susceptibility Assays

Antibacterial activity of oil basil by Agar Well-Diffusion Method (ADM) The compounds' antibacterial activity was evaluated using the agar well diffusion method. dosages of 7%, 5%, 2.5%, 1.25%, and 0.625% were used for BEO, and the plates were incubated for 24 hours under aerobic conditions at 37 °C). the results are displayed in (Table 2). Plant extracts were used to test the sensitivity of *P. aeruginosa*. All trials were repeated three times, and the inhibitory zone diameter results represented by mean standard deviation.

**Table (2): The inhibition Zone of Different Concentrations of BEO against *P. aeruginosa* isolates.**

| Isolate | Zone of inhibition (mm) at different concentrations | | | | | |
|---|---|---|---|---|---|---|
| | 7% | 5% | 2.5% | 1.25% | 0.625% | LSD |
| P1 | 21±0.4 | 18±0.3 | 16±0.3 | 13±0.5 | 9±0.3 | 6.05 * |
| P11 | 20±0.2 | 17±0.4 | 15±0.5 | 12±0.5 | 7±0.6 | 5.97 * |
| P12 | 22±0.4 | 19±0.6 | 15±0.6 | 13±0.3 | 8±0.5 | 6.33 * |
| P14 | 21±0.8 | 19±0.4 | 16±0.4 | 13±0.8 | 8±0.9 | 6.72 * |
| P15 | 20±0.3 | 18±0.5 | 15±0.8 | 13±0.3 | 9±0.4 | 5.88 * |
| P16 | 23±0.4 | 21±0.3 | 17±0.4 | 14±0.4 | 8±0.8 | 6.51 * |
| P17 | 22±0.8 | 19±0.4 | 16±0.7 | 13±0.4 | 10±0.4 | 6.38 * |
| P18 | 20±0.3 | 18±0.7 | 17±0.5 | 14±0.5 | 7±0.9 | 5.97 * |
| P21 | 21±0.5 | 18±0.4 | 15±0.4 | 12±0.3 | 9±0.2 | 6.23 * |
| P22 | 23±0.3 | 20±0.3 | 16±0.4 | 11±0.8 | 8±0.2 | 6.89 * |
| LSD | 5.92 NS | 4.79 NS | 3.59 NS | 3.91 NS | 3.27 NS | --- |
| * (P≤0.05). | | | | | | |

***Significant. NS. Nonsignificant.**

The bacterial strains tested positive for BEO's antibacterial activity. BEO exhibited antibacterial activity as well as the ability to inhibit the development of *P. aeruginosa* isolates. At 7% concentration, P16 and P22 isolates had an inhibition zone diameter of 23mm, whereas P11 and P18 isolates had an inhibition zone diameter of 7mm at 6.25% concentration. Other inhibitory diameters ranged from 20 to 9 mm for dosages of 5, 2.5, and 1.25% Because the widths of the inhibitory zone changed across dosages, so did the antibacterial activity. Although the isolates at the same concentration do not differ appreciably. These findings were consistent with those of (20), who that at concentrations of 100 to 6.25mg/ml of BEO, the average

inhibition zone against gram- negative bacteria was 22 to 7 mm, and with those of (21) discovered that *Ocimum basilicum* L essential oils exhibited high percentages of inhibition ranging from 7 to 25 mm against tested bacteria.When the antibacterial activity test results for BEO were compared to the results of antibiotic sensitivity testing utilizing the Kerby-Bauer technique, it was observed that MDR isolates were exceedingly resistant to several antibiotic agents (CLSI, 2022). Their maximum inhibitory zone was 30 mm in diameter. It was responsive to BEO, with inhibition zones extending from 7 to 25 mm, indicating that BEO has antibacterial activity against multidrug-resistant *P. aeruginosa*.

**Antibacterial activity of CSNPs loaded BEO**

The antibacterial activity of CSNPs loaded with BEO was examine using the Disc Diffusion Method, with five serial dilution doses prepared and tested on *P. aeruginosa* isolates and incubated at 37°C for 24 hours, and the outcomes are given in (Table 3).

The examined bacterial strains reacted positively to the antibacterial effect of CSNPs loaded BEO. Antibacterial activity and the ability to suppress the development of *P. aeruginosa* isolates were demonstrated by CSNPs-loaded BEO. P16 and p22 isolates exhibited an inhibition zone diameter of 28 mm at 100% concentration, whereas P11 isolates had an inhibition zone diameter of 8 mm at 6.25% concentration. Other inhibitory diameters ranged from 23 to 12 mm for concentrations of 50, 25, and 12.5 %.These findings were congruent with those of (22), who showed that the average inhibition zone of CSNPs loaded BEOs against *P. aeruginosa* bacteria was 30 to 8mm at concentrations ranging from 100 to %. Aside from chitosan's antibacterial characteristics, the addition of BEO has increased its antimicrobial function and hence hastens the suppression of bacterial development (23). The combined action of the naturally occurring compounds' oxygenated monoterpenes group and the amino or carboxyl group of the composite system may be responsible for the antibacterial activity of CSNPs loaded BEO(24).CSNPs loaded BEOs showed good physical stability. Due to the small size of the NPs, the CSNPs containing the essential oils penetrate the cell membrane of the bacteria easily. This allows the hydrophobic molecules contained in the essential oils to damage the cell, branes by altering the integrity of the phospholipid bilayer or by interfering with the active transport proteins included in the phospholipid bilayer (25) Changes in the permeability of the disrupted cell membrane cause leakage of nucleic acids, proteins, and potassium from within the bacterial cell, rendering the cell membrane unstable, thereby preventing the growth of bacterial cells. The comparison of the antibacterial activity of essential oils and CSNPs loaded BEOs shows that CSNPs loaded BEOs are much more effective. Small CSNPs can bring essential oils to the surface of bacterial cell membranes, while essential oils (which have low water solubility) cannot easily interact with cell membranes because of their larger size than that of chtosan (26).

**Table (3): The antibacterial activity of different concentrations of CSNPs loaded BEO on the number of bacterial colonies of *P. aeruginosa***

| Isolates | Zone of inhibition (mm) at different concentrations | | | | | LSD |
|---|---|---|---|---|---|---|
| | 100 % | 50 % | 25 % | 12.5 % | 6.25 % | |
| **P1** | 24±0.7 | 20±0.2 | 17±0.6 | 14±0.5 | 9±0.7 | 6.41 * |
| **P11** | 24±0.2 | 19±0.3 | 16±0.9 | 12±0.4 | 8±0.3 | 5.96 * |
| **P12** | 25±0.8 | 21±0.2 | 18±0.4 | 14±0.5 | 9±0.2 | 5.71 * |
| **P14** | 25±0.4 | 21±0.3 | 18±0.6 | 14±0.5 | 9±0.9 | 6.02 * |
| **P15** | 24±0.6 | 21±0.5 | 17±0.2 | 14±0.8 | 8±0.4 | 6.59 * |
| **P16** | 28±0.7 | 23±0.4 | 19±0.9 | 15±0.6 | 9±0.2 | 7.10 * |
| **P17** | 25±0.5 | 22±0.8 | 18±0.3 | 15±0.1 | 10±0.4 | 6.42 * |
| **P18** | 23±0.9 | 20±0.2 | 18±0.3 | 14±0.8 | 9±0.4 | 6.37 * |
| **P21** | 25±0.2 | 22±0.5 | 19±0.6 | 13±0.5 | 9±0.5 | 6.84 * |
| **P22** | 28±0.5 | 23±0.6 | 18±0.3 | 13±0.5 | 10±0.4 | 7.02 * |
| **LSD** | 5.48 NS | 4.82 NS | 4.01 NS | 3.66 NS | 3.28 NS | --- |
| * ($P \leq 0.05$). | | | | | | |

*** significant, NS: Non-significant.**

## Conclusion

The results of the present study showed that chitosan NPs loaded with essential oil of Ocimum basilicum extract have antibacterial activity against *P. aeruginosa* strains resistant to antibiotics. Considering the effectiveness of plant extracts on multidrug-resistant bacteria in vitro, we recommend further investigating these extracts as therapeutic alternatives in vivo, especially after loading with nanomaterials, as this increases their antibacterial activity

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