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Riyam Lateef Khalaf
 Ibn Sina University of Medical
 and Pharmaceutical Sciences,
 Baghdad, Iraq

Ola Abdul Kareem Nori
 Ibn Sina University of Medical
 and Pharmaceutical Sciences,
 Baghdad, Iraq

Safa Salam Abdulameer
 Ibn Sina University of Medical
 and Pharmaceutical Sciences,
 Baghdad, Iraq

Mina Mohamed Kadhim
 Ibn Sina University of Medical
 and Pharmaceutical Sciences,
 Baghdad, Iraq

Corresponding Author:
Riyam Lateef Khalaf
 Ibn Sina University of Medical
 and Pharmaceutical Sciences,
 Baghdad, Iraq

Ternary Co₃Cr₂NiO₈ nanocomposite synthesis by *Olea europaea* leaves extract and study the bacterial activity in dental decay

Riyam Lateef Khalaf, Ola Abdul Kareem Nori, Safa Salam Abdulameer and Mina Mohamed Kadhim

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Abstract

Nanomaterials have unique characteristics as compared to bulk materials. Nanomaterials have unique qualities due to their tiny size, such as increased reactivity, higher surface area to volume ratio, and enhanced structural properties. This research attempted to synthesize Cobalt-chrome-Nickel nanocomposite by using *Olea europaea* leaves extract. The synthesized Cobalt-chrome-Nickel nanocomposite from *Olea europaea* leaves was synthesized using a green method. The detailed characterization of Cobalt-chrome-Nickel nanocomposite was investigated using UV-visible spectroscopy, field emission Scanning Electron Microscopy (FE-SEM), X Ray Diffraction (XRD), FTIR and EDX. These measurements show that these Cobalt-chrome-Nickel nanocomposites were spherical in shape with a diameter of 58.15-71.92 nm and were stable for at least two months. Finally, the antibacterial activity of Cobalt-chrome-Nickel nanocomposite was determined by spread plate method and found that the synthesized Cobalt-chrome-Nickel nanocomposite has significant antibacterial activity against three bacterial species: *Streptococcus mutans*, *Streptococcus mitis* and *Staphylococcus lentus* in dental infections.

Keywords: *Olea europaea*, cobalt-chrome-nickel nanocomposite, green synthesis, antibacterial dental infections, *Streptococcus mitis*

Introduction

Dental caries, sometimes called tooth decay, are one of the most widespread chronic diseases of people in the world. It is active, reversible, preventable and complicated biofilm-mediated multifactorial disease. Caries can affect all age groups throughout the life cycle [1].

Many factors lead to the development of dental caries, such as the adhesion of bacteria with the tooth surface, biofilm formation on the teeth (dental plaque), and the local demineralization by the acidic by-products from bacterial fermentation of carbohydrates initiated on the tooth surface [2].

Caries management requires dentists to show the degree of severity factors in the treatment. Many new technologies have been applied to the treatment of dental diseases. Caries Management by Risk Assessment (CAMBRA) system developed in 2002, Prevention is always better than cure, But the activity of caries may need to be treated [3]. In general, two approaches are used to carry removal from teeth with susceptible and asymptomatic pulps: nonselective caries removal (traditional approach) and selective caries removal [4].

Biosynthesis by plant leaves is an environmentally friendly synthesizing of nanomaterials, low cost and rapid synthesis. Plant leaves act as reducing and stabilizing agents to keep nanomaterials in nanoscales for a long period of time [5]. Metal salts are mixed with plant leaves mixed under control conditions such as temperature and pH. Biomolecules reduce the metal ion to nanoscale. *Olea europaea* leaves are rich in bioactive compounds such as polyphenols, flavonoids and oleuropein. These compounds act as reducing agents [6, 7].

Joshua A. et al. in (2021) studied provides a comprehensive evaluation of current nanomaterial uses in dental fillings and procedures. The study compared standard and nanomaterial-based dental filling materials. Material comparisons consider biocompatibility,

toxicity, reactivity, cost, and antimicrobial/antibacterial characteristics. Not one material has proven to have all the beneficial qualities/characteristics desired for the filling. After analyzing three commonly used dental filling nanomaterials (Carbon-Based Nanotubes, Silica Nanoparticles, and Silver-Coated Nanoparticles), it was found that Silica Nanoparticles had the largest material benefit and should be suggested for further usage. Issues with future dental filling applications [8].

Zahra Vaseghi, Omid Tavakoli, Ali Nematollahzadeh in (2018) have evaluation of the antibacterial activity of the synthesized ternary nanoparticles and study antibacterial activity is tested against important oral strains, such as *Streptococcus mutans* associated with dental caries and *Porphyromonas gingivalis* associated with gingivitis. Evaluation is performed using laboratory tests such as the well diffusion method and the minimum inhibitory concentration (MIC). When comparing the three most currently used dental filling nanomaterials - Carbon-Based Nanotubes, Silica Nanoparticles and Silver-Coated Nanoparticles - it was observed that Silica Nanoparticles demonstrated material benefit performance [9].

The aim of this work is to synthesis eco-friendly, low cost, and rapid synthesis of ternary nanocomposite characterization nanocomposite synthesized by multiple techniques such as FTIR, XRD, FE-SEM and EDX. Apply ternary nanocomposite as antibacterial against three species of bacteria: against *Streptococcus mutans*, *Streptococcus mitis* and *Staphylococcus lentus* in dental infections

Experimental

Nanocomposite synthesis

Nanoparticles: Individually the source of metals (cobalt, copper and Nickel) were used as a stoichiometric ratio (1:1:1) to prepare ternary oxides of $\text{Co}_3\text{Cr}_2\text{NiO}_8$ Nanocomposite. Accordingly, 50 ml, 0.01 mole of each $\text{Co}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3$ and NiCl_2 were mixed at 25 °C.

To synthesize the Cobalt-chrome-Nickel nanocomposite two milliliters of *Olea europaea* leaf extract were added drop by drop to five milliliters of the stock solution of Cobalt-Copper- Nickel. The volume was filled to 50 ml with distilled water, and the sample was heated for 15 minutes at 70°C. The synthesis of Cobalt-chrome-Nickel nanocomposite is indicated by a shift in solution color from yellow to brownish yellow to deep brown. The sample was then dried, yielding the Cobalt-Copper- Nickel nanocomposite for characterization. Fourier transform infrared (FTIR) spectra were obtained at room temperature on a Shimadzu FTIR 84005 spectrometer for the plant extract containing Cobalt-chrome-Nickel nanocomposite (0.01 g) dried at 60 °C for 4 hours using KBr [10]. An X-ray Diffraction (XRD) pattern was produced using a Shimadzu XRD-6000 diffractometer with $\text{Cu K}\alpha$ ($\lambda=1.54056 \text{ \AA}$) to validate the biogenesis of nanocomposite [11]. The morphology and contact surface of the nanocomposite were

examined using a SEM S-4160 [12, 13].

Application of nanocomposite for Antibacterial activity in dental decay

1. Sample collection

Oral swab were collected from patient with dental decay who visited Ibn Sina University of medical and pharmaceutical sciences with similar oral hygiene level were selected for data collected, each carious tooth examined using a Williams periodontal probe to ensure a cavity depth of 2 mm for Standardization ;all patients examined and sampled by the same dentist [14].

2. Antibacterial activity

Studying the antibacterial activity for nanocomposite after collection and identification through the VITEK 2(bioMérieux, Inc.) system certain isolates were chosen for subsequent studies to examine the effect of the nanomaterial against *Streptococcus mutans*, *Streptococcus mitis* and *Staphylococcus lentus*. They were evaluated using method and inoculated by spread plate method. Bacteria were subculture on nutrient agar and incubated for 24 hours at 37 °C. Mueller-Hinton Agar plates infected with pathogenic microorganisms were used. Each plate included sterile paper disks 5mm in diameter that had been soaked with plant extract (control) and nanocomposite. The plates were then incubated for 24 hours at 37 °C. We measured and documented the inhibitory zones [15].

Results and discussion:

Synthesis

The FT-IR analysis was utilized to identify the different functional groups present in *Olea europaea* leaves extract [16]. Fig-1A shows a typical FT-IR spectrum for *Olea europaea* leaves extract along with a comparison with Fig-1B which shows $\text{Co}_3\text{Cr}_2\text{NiO}_8$ nanocomposite synthesized using this extract. Comparison of these two spectra indicated that the peak of synthesis nanocomposite and *Olea europaea* leaves extract have showed similar peaks with reduced intensity of OH and NH groups in the region between $4000\text{-}3000 \text{ cm}^{-1}$ with peak in the nanocomposite at ($3515, 3463, 3431, 3398 \text{ cm}^{-1}$). In the region $3000\text{-}2800 \text{ cm}^{-1}$ (C-H stretching) peaks at $2887, 2853, 2602 \text{ cm}^{-1}$ were observed. The reduced intensity may indicate partial interaction or coverage of these groups on the nanocomposite surface.

In the region $1800\text{-}1500 \text{ cm}^{-1}$ (C=O and aromatic C=C stretching) A strong carbonyl peak was observed at 1714 cm^{-1} and aromatic C=C peaks at 1627 cm^{-1} .

In the *Olea europaea* leaves extract, peaks in $1770, 1747, 1668$ and 629 cm^{-1} were observed in the nanocomposite. The shifts to higher wavenumbers (blue shift) may result from coordination between carbonyl oxygen and metal ions. Strong peaks were observed at $653, 628, 578, 541, 451 \text{ cm}^{-1}$ due to the M-O (metal-oxygen) vibrations.

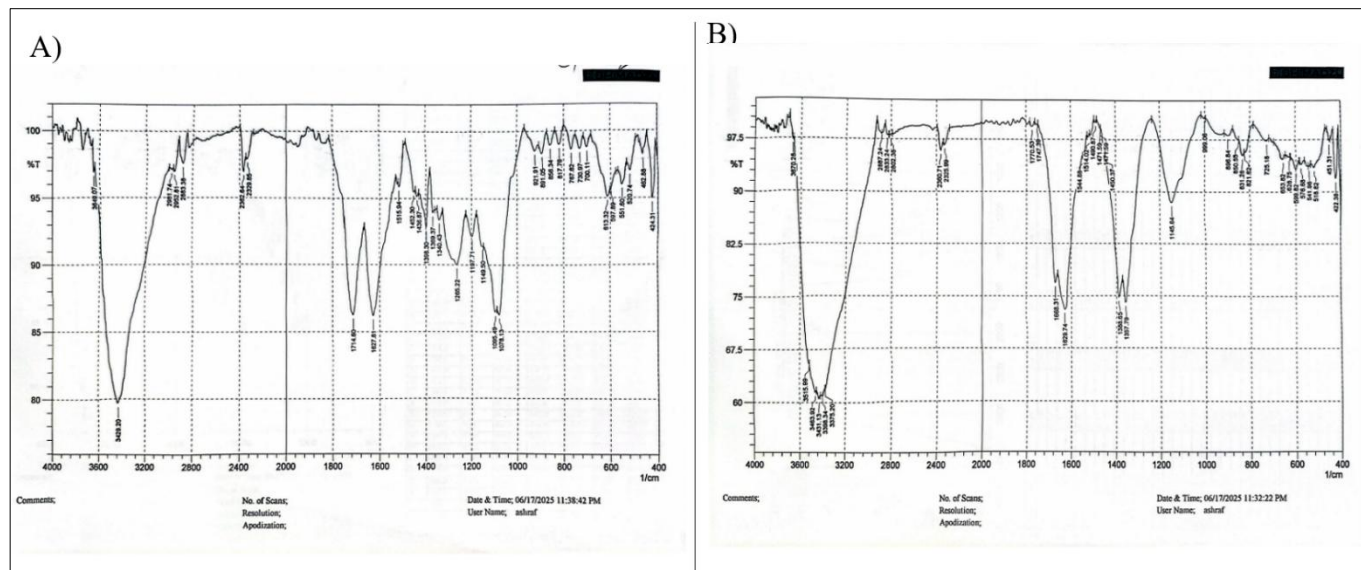


Fig 1: A) FT-IR spectrum for *Olea europaea* leaves extract. B) FT-IR spectrum of $\text{Co}_3\text{CuNiO}_8$ Nanocomposite synthesized by *Olea europaea* leaves extract

Structural, Morphological, and Optical Properties of Synthesized Nanostructures X-Ray diffraction (XRD) The structure of the samples was studied using X-ray diffraction. The SHIMADZU XRD-6000 captured all scans with a $\text{Cu K}\alpha$ radiation line of $\lambda=1.54\text{ \AA}$ in the 2θ range of 10° to 80° . The references demonstrate the patterns of the nanocomposite [12, 17]. The XRD pattern displays a considerable number of broadening lines, which are typical of nanoscale materials. The crystallite size may be estimated using the Debye-Scherrer equation [18]. The XRD pattern of

Cobalt-chrome-Nickel nanocomposite reveals four distinct peaks at $2\theta=18.27^\circ$, 21.13° , 30.06° , 35.40° , and 37.03° . These peaks correspond to the (111), (200), (220), (311) and (222) crystal planes of the hexagonal nanocomposite [19]. The data show that peak broadening indicates smaller size, indicating favorable circumstances for crystal formation. The Scherrer formula was used to determine the average crystal size of the nanocomposite, which was around 2.7 nm.

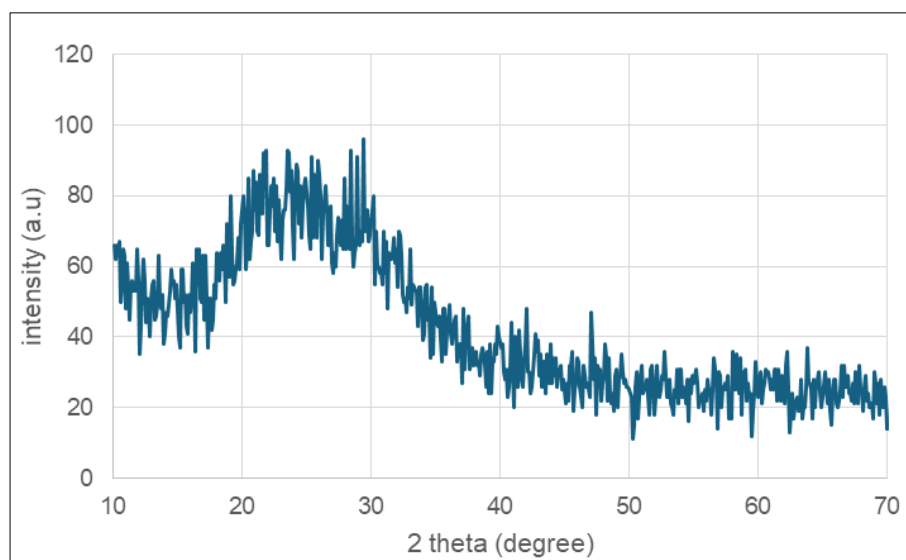


Fig 2: XRD of $\text{Co}_3\text{CrNi}_2\text{O}_8$ Nanocomposite synthesized by *Olea europaea* leaves extract

As seen in Fig. (3), the shape and size distribution of nanocomposites were examined using Field Emission Scanning Electron Microscopy (FE-SEM). Nanocomposites have a smooth, well-crystalline surface. According to the measurement pictures, the samples had a high porosity, indicating amorphous ratios. In the samples, the spherical form predominates [20]. The synthetic nanoparticles' average size ranges from 58.15 to 71.92 nm. Very small percentages

of the samples have some clusters; however this happened when the sample was being prepared for testing. The FE-SEM study indicates that the samples created spherical aggregates with a roughly homogeneous distribution. Furthermore, the crystal character of produced nanocomposites of the same size is shown. Measurement photos include a high percentage of tiny sizes and uniform diameters.

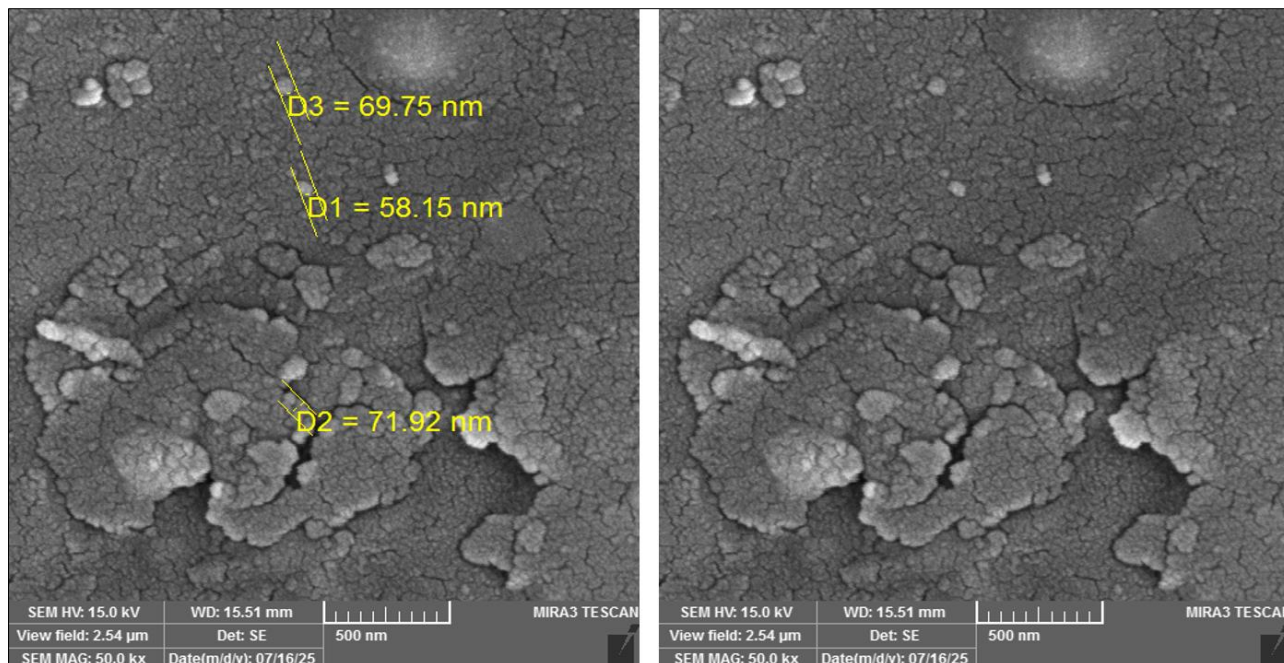


Fig 3: FE-SEM images of $\text{Co}_3\text{CrNi}_2\text{O}_8$ Nanocomposite synthesized by *Olea europaea* leaves extract

The EDX spectrum of Cobalt-chrome-Nickel nanocomposite synthesized are shown in Fig. (4) Typical Co, Ni, Cr and oxygen peaks are present in the spectrum. The results confirm a high purity of the synthesized

nanostructures [21, 22]. Furthermore, the EDX measurement which showed cobalt (54.1%), Nickel (17%), copper (20%) and for oxygen was (9.5%). The EDX of Cobalt-Copper-Nickel nanocomposite synthesized shown in fig. (4).

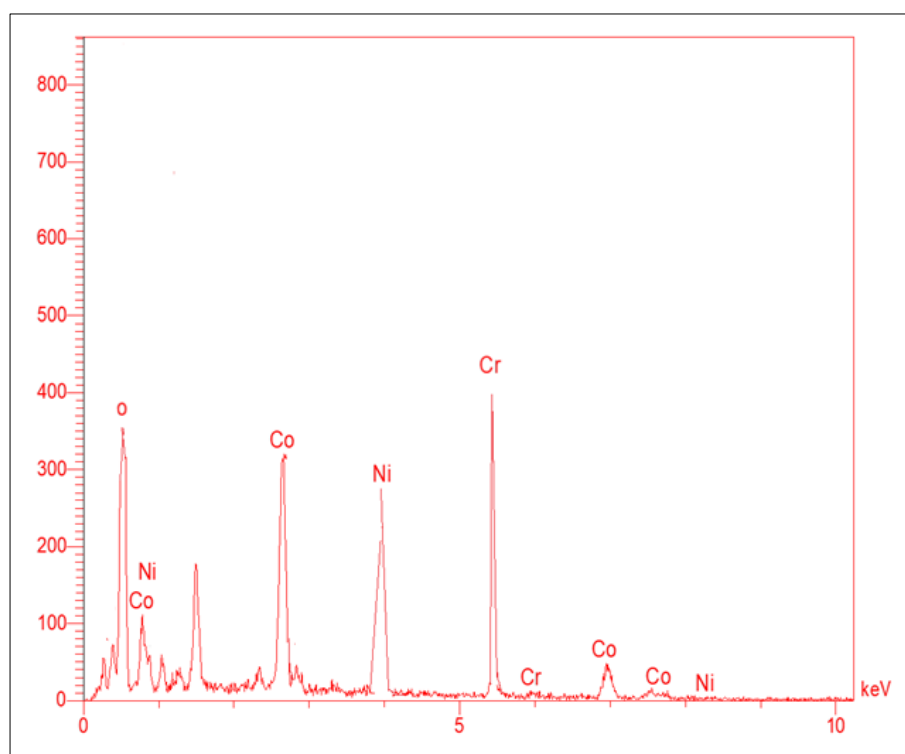


Fig 4: Image EDX of $\text{Co}_3\text{CrNi}_2\text{O}_8$ Nanocomposite synthesized by *Olea europaea* leaves extract

Antimicrobial assay of Cobalt-chrome-Nickel nanocomposite: In the present study, the antibacterial effect of nanoparticles was tested against *Streptococcus mutans*, *Streptococcus mitis* and *Staphylococcus lentus* [23]. Diagnoses bacteria by The Vitek analyzer were found to be valuable for the speed and accuracy with which results were available for the common bacterial pathogens [24]. The nanomaterial tested in this study exhibited varying levels of

antibacterial activity against the three isolated bacterial species, depending on the concentration used. The results on *Streptococcus mutans* showed clear sensitivity to the nanomaterial at concentrations of 50% and above. At 100%, a 20 mm inhibition zone was observed. At 75% a 16 mm inhibition zone was observed. At 50%, the inhibition zone was 12 mm, showing moderate effectiveness. No inhibition

was observed at 25%, suggesting this concentration is insufficient to affect the growth of *S. mutans*.

These findings indicate that this nanomaterial starts to be efficient at 50% and more potently at higher concentrations. In the case of *Streptococcus mitis*, the same trend was followed by *S. mutans*, but the sensitivity was a bit weaker in the intermediate concentrations. The inhibition zone was 15 mm at 100% which indicates good antibacterial activity. A 12 mm zone was seen at 75% and 10 mm zone at 50% with moderate inhibition. There was no inhibition at 25 percent meaning that there was low activity at this concentration. The progressive growth in the inhibition zone is a confirmation that the effect of the material improves with concentration [23]. In the case of *Staphylococcus lentus*, it was more resistant to the nanomaterial than *Streptococcus* species. A 14 mm inhibition zone was obtained at 100% and this shows good sensitivity with maximum concentration. A 10 mm zone was observed at 75%, but not at 50% or 25%. The findings indicate that the nanomaterial needs to be used in higher concentrations to inhibit *S. lentus*, perhaps because of the differences between the cell wall structure or

resistance strategies. One-way analysis of variance (ANOVA) was used to analyse the experimental data to determine the effect of various concentrations on the measured response. Descriptive statistics revealed a gradual decrease in mean values with decreasing concentration, where the highest concentration (1) exhibited a mean of 22.35 ± 1.79 , followed by 0.75 (19.90 ± 1.68), 0.5 (17.45 ± 1.79), and 0.25 (11.85 ± 2.23). The results of the ANOVA showed that there was a very significant difference between the groups that were tested ($F = 113.79$, $p < 0.001$) and that the difference in concentrations did have a statistically significant effect on the measured response. The result of this finding supports the existence of a robust dose-dependent correlation between concentration and activity. The high intensity of the decrease in response with low concentrations points to the fact that the performance of the tested system is directly dependent on the concentration levels. Additional post hoc test should be conducted to establish the particular differences between individual groups.

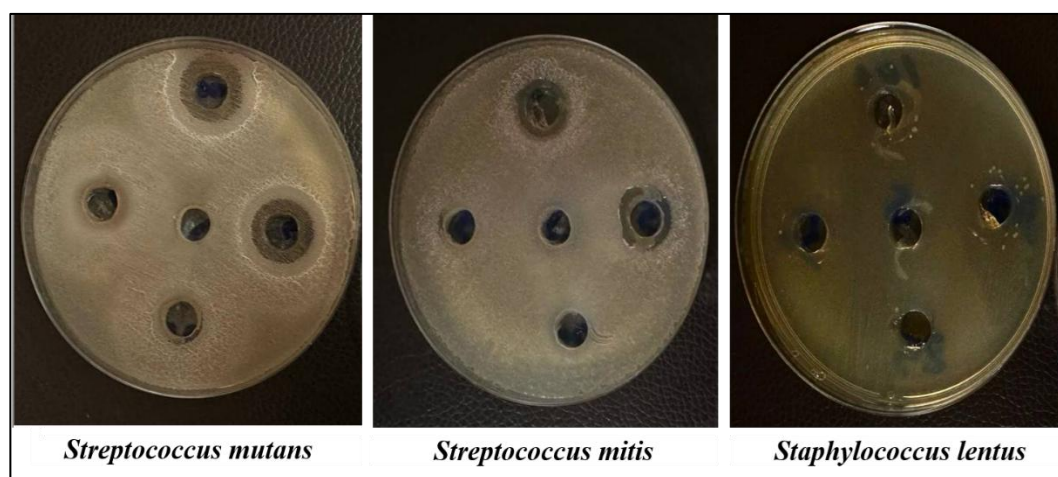


Fig 5: Effect of Nanocomposite extract against different bacterial.

Table 1: Descriptive Statistics (Mean±Standard Deviation) of Measured Response across Concentrations

Concentration	Mean±SD	Minimum	Maximum
1.00	22.35 ± 1.79	19	26
0.75	19.90 ± 1.68	17	23
0.50	17.45 ± 1.79	14	20
0.25	11.85 ± 2.23	8	17

Conclusion

A nanocomposite was successfully prepared by green synthesis, and the method is considered cheap, safe, easy to use, and fast to produce. The nanocomposites were characterized by several techniques, the most important of which are FTIR, XRD, FE-SEM and EDX. Comparison of these two spectra indicated that the peak of synthesized nanocomposite and *Olea europaea* leaves extract are similar peaks but with reduced intensity in the coordination bands of OH and NH groups. The reduced intensity may indicate partial interaction or coverage of these groups on the nanocomposite surface. The crystallite size of nanocomposite was 2.7 nm calculated from XRD analysis, the nanoparticles size was found to be 58.15 to 71.92 nm based on FE-SEM. The difference between these numbers is the larger particle size compared to the crystallites; size indicates that the nanoparticles are composed of several

crystallites, forming polycrystalline aggregates rather than single crystals. The antimicrobial evaluation of the $\text{Co}_3\text{CrNi}_2\text{O}_8$ Nanocomposite against *Streptococcus mutans*, *Streptococcus mitis*, and *Staphylococcus lentus* revealed a concentration-dependent inhibitory effect. *S. mutans* showed the highest sensitivity to the nanocomposite, with a noticeable inhibition starting at 50%, and a pronounced effect at 75% and 100%, indicating strong susceptibility. Overall, the results indicate that the synthesized nanocomposite is more effective against oral streptococci than *S. lentus*, and its antibacterial efficacy clearly enhanced with increasing concentration. This highlights its potential as a concentration-sensitive antimicrobial agent for dental or biomedical applications.

Ethical approval

The Ministry of High Education of Iraq, Ibn Sina University of Medical and Pharmaceutical Sciences approved this research.

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