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Green Synthesis of Zinc Oxide Nano Composites From Curcumin and Evaluation of their Inhibitory Activity on Porphyromonas Gingivalis Isolated From Gingivitis

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Abstract: The current study concluded the testing of antibacterial effect of compounds Curcumin besides the Curcumin/ zinc oxide nanoparticles. The bacteria P.gingivalis was isolated from Gingivitis infections and 48 (of overall 96 cases) were chronic infections and only 2 (of 4) were end stage cases. The depth of the pocket (DP) ranged between 3- 9 mm is distributed between an average of 5.03 mm in chronic infections and 3.5 mm in advanced infections. It was diagnosed by phenotypic and biochemical approaches. Curcumin/ zinc nano-composite was prepared by the green synthesis method. Then scanning election microscope (SEM), and X-Ray Diffraction XRD methods were also used. The minimum inhibitory concentration (MIC) testing was conducted using the liquid dilution method, revealing that the MIC of curcumin for the examined bacteria was 250 and 500 µg/mL, but the Cur/ZnO nanoparticles exhibited MIC values of 1000 and 2000 µg/mL.

Keywords: ZnO, green nanoparticles, curcumin, Porphyromonas gingivalis.

Introduction

Porphyromonas gingivalis is an important bacterium plays a critical role in the occurrence and development of periodontal infections. Chronic ones (Pejcic et al 2011), which are non-motile bacteria, have a wide range of production of active virulence factors that contribute to infection and destruction of gingival tissue. In addition to protecting the host's defence, the bacterial isolates have been obtained from specimens taken from subgingival pockets in patients with inflammation. around the teeth, which supports its role in these infections (Loo *et al*., 2009).

Spore-forming, gram-negative, produces black brown pigment of porphyrin, obligate anaerobic bacteria. Periodontal infections are defined as complex and multifactorial disorders that occures in interactions between host cells and anaerobic gram-negative bacteria, which are considered among the most widespread bacteria spreaded in the world. The incidence of it estimated at between 10-15% among people globally, which, if remain untreated, the tooth loss and other systemic complications are resulted (Gaentsch, 2009). In addition, these infections are related to cardiovascular diseases, bacteremia, and premature birth in pregnant women for premature babies delivery (Pejcic *et al*., 2011).

Nanoparticles of some prepared materials have unique physical and chemical properties compared to their natural size, which qualifies them to be contibuted in many fields starting from electronics and not ending with the medical field (Ahamed *et al.*, 2016).

Due to their high responsiveness and increased specific surface area, many metals and nano-elements that are considered harmless for microbes in their normal condition have demonstrated severe toxicity at the nanoscale level (Emami-Karvani and Chehrazi, 2011). Certain plant components as well as their alcoholic and aqueous extracts, have also been utilized as medicines and antibiotics. Turmeric is a plant-based spice that has medicinal qualities and is generally useful in treating bacterial infections (Loh et al., 2000).

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It is one of the most commonly used plants in medicine because it contains antioxidants and phenolic chemicals that help prevent cancer, cure ulcers, relieve pain, and treat a variety of bacterial and fungal infections. Additionally, turmeric has a wide range of antibacterial properties.

Some studies have shown its effectiveness against fungi and a number of Gram-negative and positive bacteria (Tyagi *et al*., 2015

Material and methods

January through May of 2023 saw the completion of the present study on Diyala province. The swabs have been incubated for twenty-four hours at 37 degrees Celsius in trypton soy broth (Himedia-India). The isolated bacteria were incubated for twenty-four hours at 48 degrees Celsius after being subcultured on MacConkey agar, Mannitol salt agar, Nutrient agar, EMB agar, Chocolate agar, and Blood agar. Incubation conditions and culture medium were the same for the single colony's purification. In addition to biochemical assays, the isolates were identified using Gram stain [Kwon et al., 2021].

Salivary samples from 150 periodontally healthy or diseased participants (ages 6 to 70) made up the samples used in this investigation. When there were no indications of periodontal problems, a person's periodontal condition was considered good. A clinical and/or radiological investigation revealed the existence of periodontal breakdown, leading to the diagnosis of periodontitis. Slots (2017) used the American Academy of Periodontology's standards to further categorize periodontitis.

The procedure outlined by Bashi et al. (2013), with minor changes, was used to generate nanoparticles with layers of zinc oxide and curcumin. One gram of curcumin was added to the zinc oxide solution that was created by dissolving one gram of zinc oxide in fifty milliliters of deionized distilled water. The samples were transported to the University of Kashtan in the Islamic Republic of Iran for analysis, were the hybrid nanocomposites were identified. utilizing prior to and following the injection process. The characterization of the nano-composite was done using an X-ray diffraction microscope.

Since:

n: The crystal plane order from which the X-ray diffraction takes place.

λ: The X-ray wavelengths utilized in a copper tube

ğ: the X-rays' diffraction angle.

d. The crystal distance between two parallel planes that is perpendicular (Modena and others, 2019).

The surface morphology of the two nanocomposites was determined using a scanning electron microscope (SEM). The dispersed and dried nanoparticles were placed on the top of an aluminum substrate containing a double conductive carbon ribbon. The stick was covered with a flame under an argon atmosphere using spray paint (Petal & Parsania, 2018).

The samples were then sent to Kashan University in the Islamic Republic of Iran for examination.

The disk diffusion test method was used to determine the sensitivity of the studied bacteria to the nanocomposites and Cur/Zno nanoparticles, according to what was stated in (Morello *et al*., 2006).

According to (Morello et al., 2006), the disk diffusion test method was performed to ascertain the isolated bacteria's sensitivity to antibiotics.

The image below shows the spectrum of zinc oxide; the curcumin did not form crystals and was unclear between the layers, but they are very clear with respect to zinc, and they are at the following angles: 56.71°, 52.33, 47.81°, 34.73°, 67.36°, 62.33°, with crystallographic distances (536.37, 213.27, 16.52, 221.75, 1000.00 nm 375.84), respectively, and there is one angle indicating curcumin °2 6.23 and at a distance. 38.14 nm, and the results obtained agree with Annaraj *et al* (2015).

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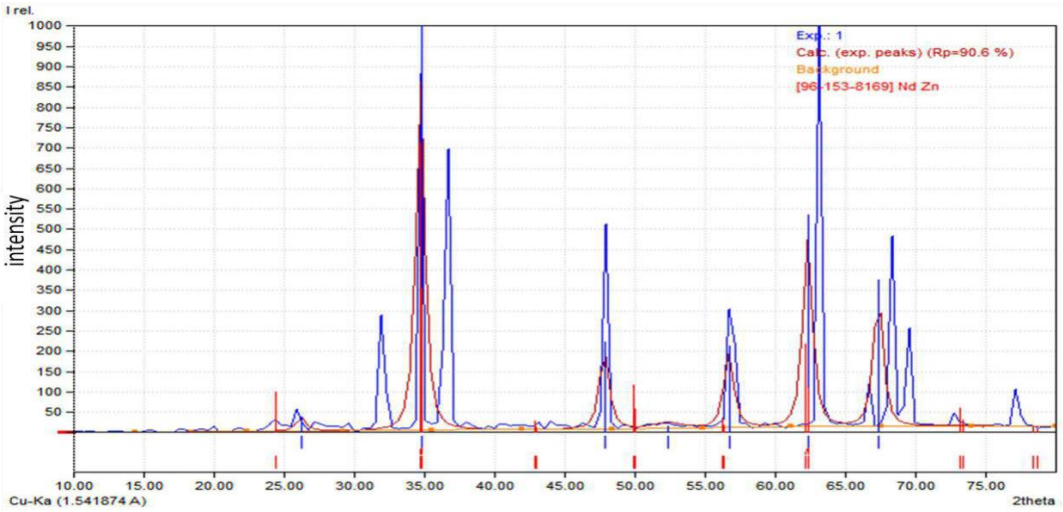


Figure (1): Scanning electron microscope (SEM) of the Cur/Zno nanocomposite.

The figure below shows how the ZnO hexagonal crystal looks. It was not quite evident, but it looked like layers of zinc oxide, possibly with curcumin in between. Above the surface, well organized zinc oxide grains were visible, which was in line with Khalil et al. (2014)'s findings. The dimension of a single molecule that was trapped within two lines was determined. It is 61.32 nm at d1, 26.49 nm at d2, and 17.37 nm at d3, suggesting that a reduction has taken place within the presence of varying-sized zinc oxide particles. This suggests that curcumin has reduced zinc oxide (ZnO) to varying-sized particles.

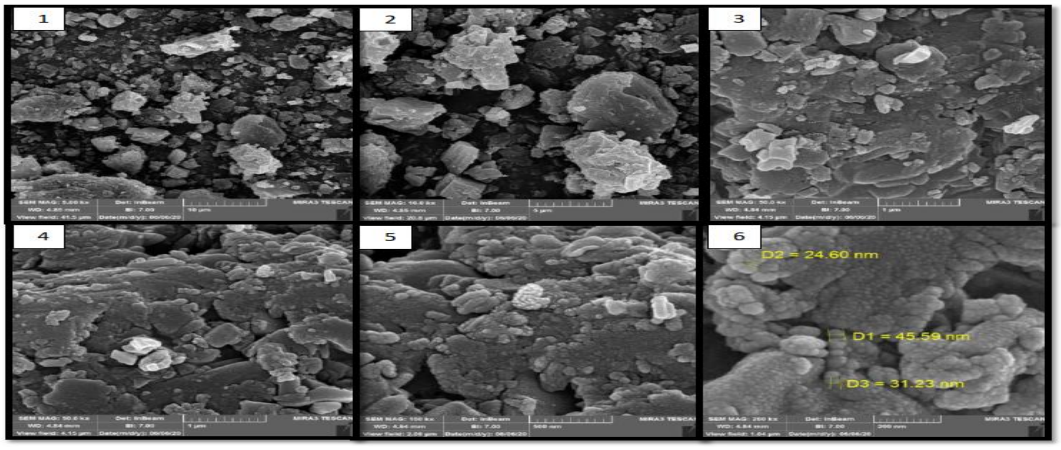


Figure 2: The ZnO hexagonal crystals

Bacterial isolation:

A sum of 150 samples were collected from periodontitis patients. After microbiology studies, the results showed positive and negative bacteria for different gram stains, but the type of study determined that of 50 isolates, 48 (96) which were all chronic infections and 2 (4) were advanced infections, and the depth of the pocket (DP) ranged between 3- 9 mm is distributed between an average DP of 5.03 mm in chronic infections and 3.5 mm in advanced infections.

The predominance of the chronic injuries over advanced cases was similar to what researchers mentioned (Salari and Kadkhoda, 2004) in their findings as the most periodontal injuries were of the chronic type and the bacterial isolates are mixed and highly virulent, in addition to they pointed out that there are many factors responsible for this complexity, including the interaction between bacterial types with the bacterial community aquired isolates and the predisposition of the host. In a previous

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study, researcher Armitage (1999) stated that periodontal infections are chronic in nature and can be resistant in the absence of treatment. That periodontal infections are chronic in nature and can be resistant in the absence of treatment.

Regarding the depth of the pocket (DP), the results of this study were consistent with what the researcher (Pfister, (2002) indicated that the depth of the pocket ranged between 4-9 mm, while researcher Kumar and his group (2003) indicated that the average depth of the pocket in their study was 7.7 mm. In previous study, researcher Kumar and his group (2005) found that 30% of adults had periodontal injuries in 3 or more teeth with a pocket depth of ≤ 4 mm

The positive association of anaerobic bacteria with deep pockets is due to the low level of oxygen in these pockets, which makes them a suitable environment for the presence of anaerobic bacteria. This was confirmed by researchers Lai and Chu (2008) who stated that the tension of oxygen 2PO in the deep pockets ranges between 5-27 mm Hg. It is much lower than in shallow pockets.

Results

Results of investigation of P.gingivalis

First: by the anaerobic culture method: After inoculating the samples from the tubes of the carrier medium into the tubes of the liquid isolation medium supplemented with BHI and incubating them anaerobically in the presence of the resazurin reagent, the presence of positive growth was observed indicative of the formation of turbidity in the medium. When plates of fortified BHI medium with 2% agar were inoculated and incubated under anaerobic conditions, distinct colonies appeared with a brown-black hue, with the growth of other colonies. Then it was purified until a pure culture was obtained, and when it was stained with gram stain and examined with a light microscope, its cells appeared to be short bacilli, negative for gram stain.

These results were consistent with what was reported by many studies. Researchers (Leon et al., 2007) described them as Gram-negative bacteria, non-motile and anaerobic. Their colonies in the middle of blood cells appear as spots. It is black-coffee as it contributes to the transport of iron using hemin as a means of transporting it, so it produces a black pigment. While the researchers (Shelburne *et al*., 5002) indicated that the production of black pigment by the P.gingivalis bacterium is due to the accumulation of hemin, which it uses as a source of iron for its growth. This may be the reason that people who consume large amounts of iron in their food have We are more susceptible to gingivitis and inflammation of the tissues around the teeth.

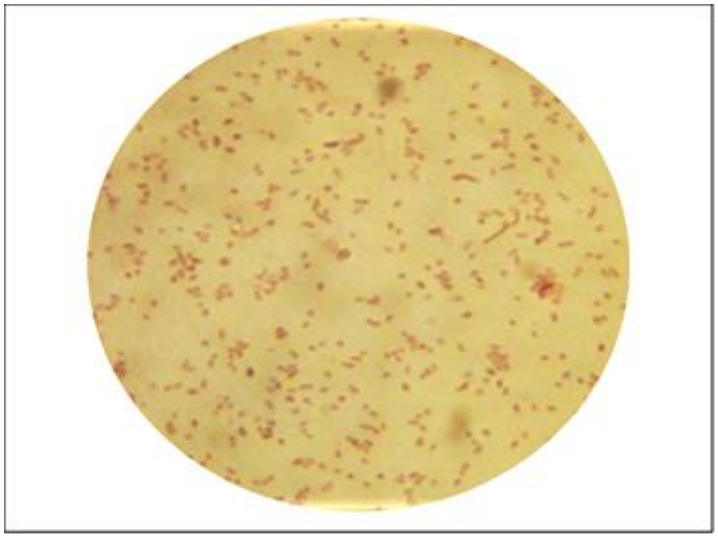


Figure (3):. *P. gingivalis* cells stained with Gram stain.

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Minimal inhibitory concentration (MIC)

We employed six different nanocomposites at µg/mL (62.5, 125, 250, 500, 1000, 2000). The minimum inhibitory concentration (MIC) for bacteria using the broth dilution method were 250 µg/mL of free curcumin. According to one study, curcumin's MIC in its single form is between 125 and 250 µg/mL.

Applying the liquid dilution method, the MIC test revealed that the bacteria under investigation had a minimum inhibitory concentration of 250 and 500 µg/mL g for curcumin, whereas the Cur/zno nanoparticles produced between 1000 and 2000 µg/mL. According to another study, curcumin's minimum inhibitory concentration (MIC) on bacteria was 219,217 µg/mL. (2013) Gunes et al. Regarding the Cur/Zno nanoparticles, they are absent. Give the bacteria 1000 µg/mL. According to earlier research, the bacteria according to investigation has a MIC ratio of 1000 µg/mL for Cur/Zno NPs (Perera et al., 2020).

Testing the sensitivity of P.gingivalis isolates to nanocomposites:

Free curcumin affects P. gingivalis bacteria, according to the data in the table below. Since A. is one of the bacteria that is most affected by curcumin, it suppresses it well at a concentration of 13.7 mM and coincides with the findings of other studies (Teow et al., 2016). Additionally, curcumin's ability to weaken the bacterial membrane, regardless of its condition with regard to the gram stain, and cause its breakdown has demonstrated its antibacterial action (Tyagi et al., 5102).

Specifically, the concentration was 1 M of curcumin to 1 M of zinc oxide while creating the nanomaterial. Two times as much free curcumin was present as in the Cur/Zno nanocomposite. As a result, the nanomaterial performs better than the free substance. The high findings of inhibition of Cur/Zno nanoparticles suggest that curcumin and zinc oxide work in concert, making this combination suitable for application as an antibiotic.

Whenever used as a medicinal medication, the average diameter of the inhibitory zone on P. gingivalis bacteria was 15.4 and 17 mm at 5 mg/ml. Due to its hydrophobic nature, curcumin binds firmly to the bacterial membrane, increasing the starch and effectiveness of nanocomposites (Snigdha et al., 7102). The primary processes suggested for Cur/Zno nanoparticles are the release of zinc (Zn) ions and the production of reactive oxygen species, which kill bacterial cells. They might cooperate to create an inhibitory and antibacterial impact (Snigdha et al., 2017).

Table 1: The effectiveness of nanocomposites

Bacteria	Subject	Concentration (mg/ml)	Damping diameter (mm)
p.gingivalis	curcumin	**2**	**13.77±1.17**
	curcumin/zno NPs	**2**	**8.13±0.97**
		4	**10.12±1.09**
		6	**12.06±1.05**
		8	***13.87±0.97**

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