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# Green Synthesis of Nanocomposite: Based on [Eugenol and Metal Oxides], Characterization and Biomedical Applications

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## Abstract

Eugenol (EUG) was reacted as a matrix with a mixture of metal oxides (ZnO and CuO) have been prepared from metal acetate, Zn $(CH_3CO_2)_2$ and Cu $(CH_3CO_2)_2$ as precursors and using ethylene glycol (EG) as a solvent for synthesis the [Eug/ ZnO: CuO] nanocomposite using Sol-Gel method. The synthesized nanocomposite was characterized using techniques (FT-IR, AFM, SEM, EDX, and XRD), where the average particle diameter was found to be within the nanoscale range. It was also observed that the prepared nanomaterial was in the form of rods with good homogeneity distribution. In order to stand on the obtained nanoscale properties, those properties were employed in terms of nanoscale dimension and shape characteristics, to investigate the effectiveness of the prepared nanocomposite [Eug/ ZnO: CuO] as antimicrobials (antibacterial and antifungal) activity against two types of bacteria *[Escherichia Coli (-) (E. coli), and Staphylococcus aureus (+) (S. aureus)]*, and one type of fungi *[Candida albicaus (C. albicaus)]*, where it showed acceptable results. The effectiveness of the prepared nanocomposite was also measured as the antioxidant against free radicals and it showed a good scavenging ratio. In addition, the cytotoxic effect of [Eug/ ZnO: CuO] nanocomposite on breast cancer cells (MCF-7) was studied, and it showed acceptable results in killing the cell line (MCF-7) at high concentrations.

**Keywords:** Biomedical, Eugenol, Green synthesis, Metal oxides, Nanocomposite.

## Introduction

Eugenol (EUG), also known as 4-allyl-2-methoxyphenol, is a phenylpropanoid with a substituted allyl chain for guaiacol Fig. 1 [1]. The name is taken from Eugenia aromaticum, also known as Eugenia caryophyllata, which is the scientific name for clove [2] and it's traditionally produced from the dried flower buds of Eugenia caryophyllata Thunb (Myrtaceae) [3]. Also, it is a naturally occurring compound that has been found in various plant groups 1. It is transparent to light yellow greasy liquid. It is the primary component of clove essential oil [2], it makes about 83–95 percent of clove oil [4]. It's the most volatile, biologically active component, and gives cloves their characteristic scent [2,3]. It has a low chemical stability, is susceptible to oxidation, and is soluble in tiny amounts in water but readily dissolves in organic solvents [5]. (EUG) is an antimicrobial phenolic component of cinnamon essential oil and is well-known for its anti-inflammatory, antioxidant, anticarcinogenic, anti-virus, anti-bacteria, anti-coagulation, anti-platelet aggregation, and analgesic properties [6,7]. Due to the presence of many functional

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groups, including allyl ($-CH_2-CH=CH_2$), methoxy ($OCH_3$), and phenol (OH) [8].

Synthesis and characterization of nanocomposites received significant research interest because they have a much more surface area than their bulk counterparts, which gives them special features and possible applications [9] such as medical treatments, civil, health, fabrication, information, techniques, environments, and energy sources [10]. Nanocomposites are a combination or matrix, when several materials are combined to create novel material characteristics, one of the components must have a size in the range of 1-100 nm [11]. A nanocomposite is a solid material composed of many phases, at least one of which has one, two, or three dimensions, each with a nanometer-sized dimension [12]. Metal oxide nanocomposites (MONCs) production is currently receiving more and more attention. The primary cause of this is the broad range of applications for such materials, which include fuel cells, photovoltaics, cosmetics, medicine, semiconductor packing materials, water treatment, and catalysts [13]. Due to their size, stability, high surface area, catalytic activity, simplicity in fabrication, and selectivity for particular reactions [14,15], (MONCs) have been used in a variety of chemical reactions, including coupling reactions, electrochemical reactions, as well as oxidation and reduction reactions [15]. (MONCs) with various morphologies have been created by physical, chemical, and biological processes [9,14], such as sol-gel, hydrothermal, co-precipitation, thermal decomposition, microemulsion, solvothermal, microwave-assisted, and sonochemical [16]. In our research, we employed a sol-gel preparation approach. The sol-gel approach has shown to be versatile and has been widely employed to prepare organic/inorganic hybrid materials because of its high product purity and homogeneous composition[17]. The reactions are carried out using the sol-gel approach at low temperatures or room temperature, and it is inexpensive and simple to utilize [18]. The present work's objective is to synthesize zinc copper oxide ZnO: CuO with eugenol as a nanocomposite via the sol-gel method and study biological applications through antimicrobial, antioxidant, and anticancer activities

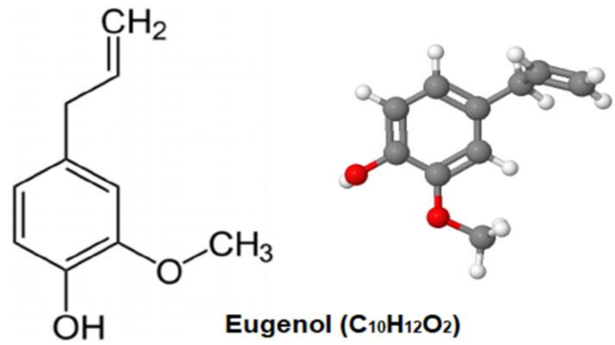


**Figure 1. The structure of Eugenol used in this study**

## Materials and Methods

Two salts, (0.01mol) of Zn $(CH_3CO_2)_2$ and (0.01mol) of Cu $(CH_3CO_2)_2.6H_2O$ were dissolved in 40 ml of ethylene glycol (EG). Then 1ml of eugenol was added to this mixture. After that was stirred magnetically for (2 hrs) at room temperature and then the obtained (gel) was dried in the evaporating crucible on the hot plate at (150 °C) for (1hrs) to obtain the [Eug/ ZnO: CuO] (NC). After that, the separated sample was dried at (75 °C) for three days.

**Chemicals Used:**

All of the organic compounds used in the present research were of the greatest purity possible.

**Instrumentations:**

Different apparatuses were used in this work for the characterization of the synthesized nanostructures as illustrated briefly below, in Table .1.

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**Table 1. Instruments utilized in this current project.**

| Instrument | Specification | Laboratory | Function |
|---|---|---|---|
| **Atomic force Microscopy (AFM)** | NaioAFM2022, Nanosurf AG Switzerlancl | Chemistry Analysis Center/ Baghdad | Morphology of coated surfaces |
| **Scanning electron microscopy–energy dispersive X-ray (SEM-EDX)** | TESCAN-MIRA3 Czech Repblic | University of Tabriz/ Iran | Morphology of coated surfaces |
| **X-ray diffraction (XRD)** | ADX 2700 Angstrom Advanced Inc. USA | University of Kashan/ Iran | Crystals structure & Type of phase |
| **Fourier transform Infrared spectroscopy (FT-IR)** | SHIMADZU FT-IR 8400S/Japan | Chemistry department, College of Science, University of Baghdad | Chemical Compounds |

## Results and Discussion

### FT-IR

Fig. 2 reveals a peak at 516 $cm^{-1}$ that was assigned to the stretching vibration of (Zn-O) bond [19] and the broad absorption band at 3452 $cm^{-1}$ attributed to the stretching vibration of Eugenol (O-H) group [20-22]. The spectrum shows peaks at 613, 661, and 694 $cm^{-1}$ confirming the formation of (Cu-O) [23]. The (C=C) vibrations of the benzene ring were revealed at 1508 $cm^{-1}$ [20-22]. The weak intensity band at 1236 $cm^{-1}$ was assigned to the (C-O) vibration of Eugenol [20-22].

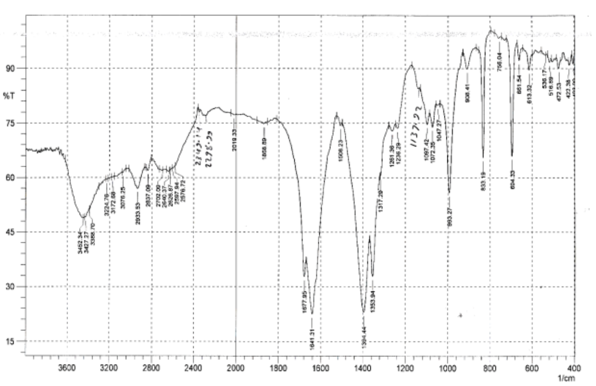

**Figure 2. FTIR spectrum of [Eug/ ZnO: CuO] nanocomposite.**

### AFM

Fig. 3 shows the surface topography (roughness) of the [Eug/ ZnO: CuO] nanocomposite prepared by the sol-gel method. The results showed that (Ra) is 47.66 nm and (Rq) is 61.93 nm. Nanoparticle mean diameter ranged from 25 to 125 nm, with many smaller than 25 and 50 nm.

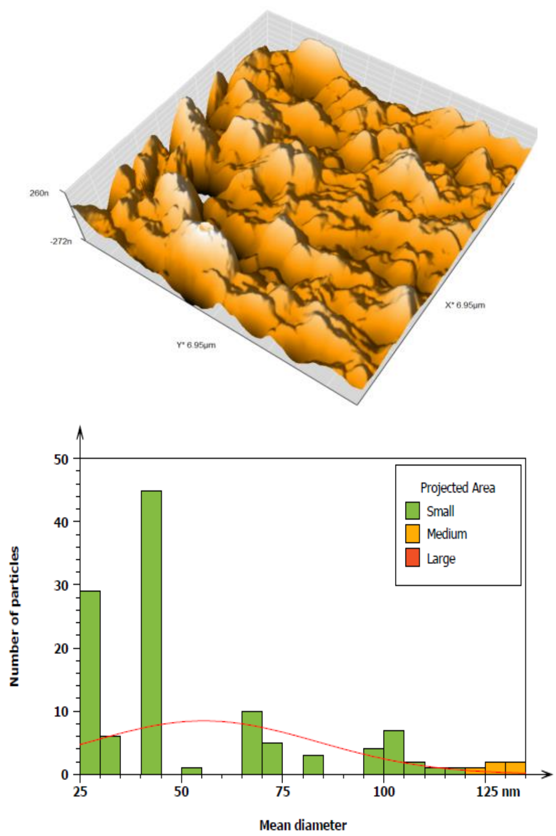


**Figure 3. AFM analysis image and particle size distribution of the [Eug/ ZnO: CuO] nanocomposite.**

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## SEM & EDX

Fig. 4 (A, B) shows the SEM image of [Eug/ ZnO: CuO] by different magnifications. Nanorods of materials with a good homogenous distribution have been shown, the sol-gel method for prepared [Eug/ ZnO: CuO] can be considered more active and novel to prepared nanorods by different diameter, the diameter of the nanorod around 98 nm. Figure 5, illustrates the EDX analysis of (Zn, Cu, and O) and explained the total percent about 63.04%, and the other percent return to the carbon of eugenol as a binding material.

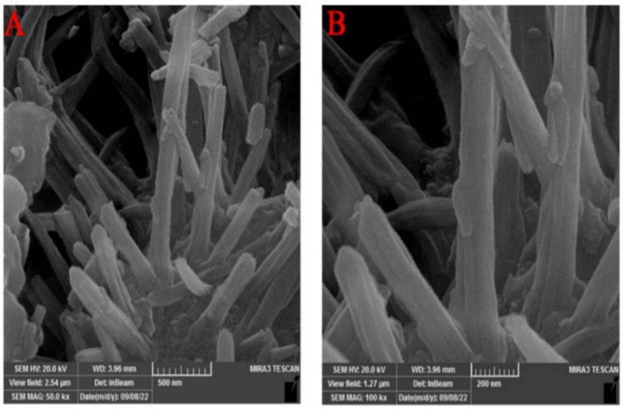


**Figure 4. SEM Images (A, B) of [Eug/ ZnO: CuO] with different magnifications 50Kx, and 100 Kx, respectively.**

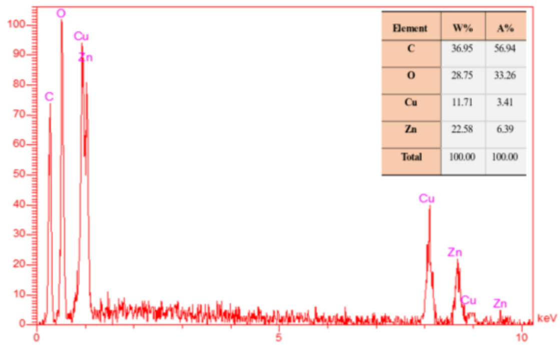


**Figure 5. EDX chart of [Eug/ ZnO: CuO] nanocomposite.**

## XRD

Fig. 6, illustrates the pattern of XRD for [Eug/ ZnO: CuO] NPs prepared by sol-gel method, as seen there is good crystallinity of the nanomaterials prepared and multi peaks agreement with astandard card of ZnO-Cu according to JCPDS matched by (00-006-0657)[24] by using Debye Scherrer's formula [25], the mean crystallite size has been calculated to be approximately 58.5 nm. Table 2, explains the FWHM and Crystallite size of [Eug/ ZnO: CuO].

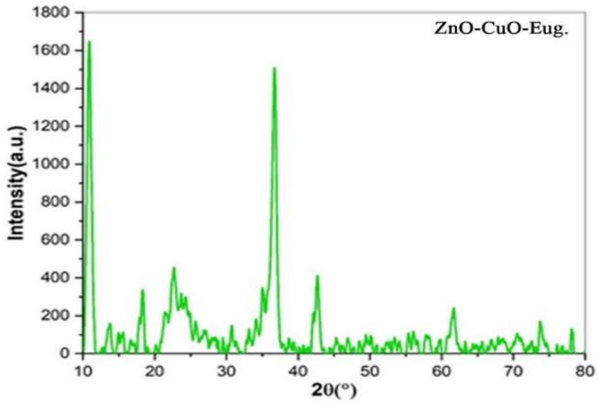


**Figure 6. XRD pattern of [Eug/ ZnO: CuO] nanocomposite.**

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**Table 2. Explain the FWHM and Crystallite size of [Eug/ ZnO: CuO] nanocomposite.**

| Nanocomposite | The highest peaks refer | 2theta (deg.) | d-spacing [Å] | FWHM (deg.) | 2theta (Rad.) | FWHM (Rad.) | D (nm) | Matched by |
|---|---|---|---|---|---|---|---|---|
| [Eug/ ZnO: CuO] | ZnO-Cu | 42.6618 | 6.2432 | 0.2952 | 0.745 | 0.005 | 36.582 | 00-006-0657 |
| | | 78.1506 | 3.36428 | 0.48 | 1.364 | 0.008 | 80.57 | |

## Applications

### Antimicrobial Activity

The microbial activity of synthesized nanostructure as nanocomposite has been tested against two bacteria *[Escherichia Coli (-) (E. coli), Staphylococcus aureus (+) (S. aureus)]* and fungal *[Candida albicaus (C. albicaus)]*, it took (0.06 gm) from the [Eug/ ZnO: CuO] nanocomposite sample then, dissolved in 5ml of dimethyl sulfoxide (DMSO) and the direct inhibitory effect of [Eug/ ZnO: CuO] against pathogenic microorganisms was determined by well diffusion method under aerobic conditions[26]. As shown in Fig. 7- 9 and Table 3. It has been observed that the levels of antibacterial and antifungal activities of [Eug/ ZnO: CuO] at the highest concentration of nanoparticles were the largest zone of inhibition 17 mm against (*E. coil*) and (28 mm) against (*S. aureus*) for bacteria and 20 mm against (*C. albicaus*) for fungal, compared with the used antibiotic (amoxicillin), which the zone of inhibition was less.

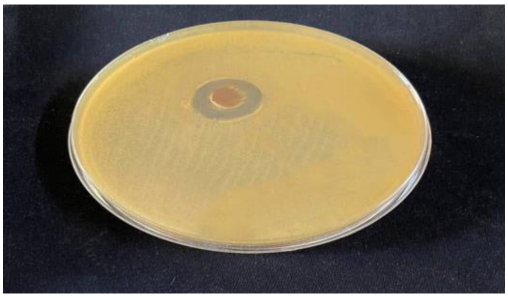

**Figure 7. Zones of inhibition of bacteria activity of [Eug/ ZnO: CuO] nanocomposite on *E. Coli (-)*.**

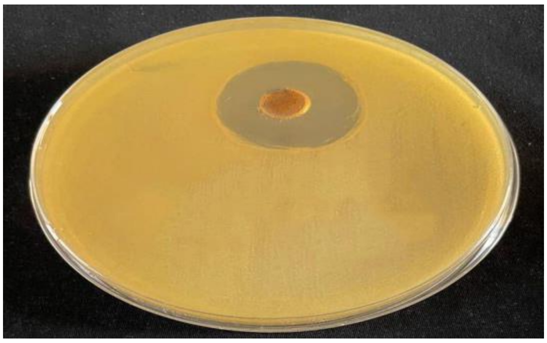

**Figure 8. Zones of inhibition of bacteria activity of [Eug/ ZnO: CuO] nanocomposite on *S. aureus (+)*.**

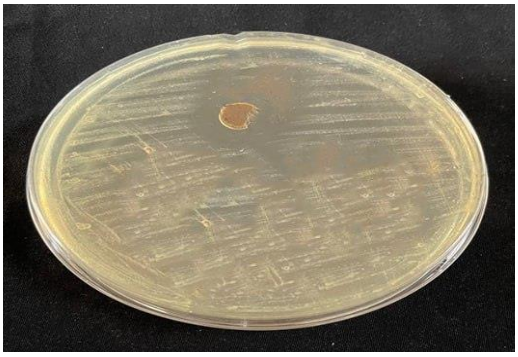

**Figure 9. Zones of inhibition of the fungal activity of [Eug/ ZnO: CuO] nanocomposite *on C. albicaus*.**

**Table 3. Inhibition of pathogenic many bacteria and fungi on nanocomposite.**

| Nanocomposite | *E. coli (-)* (mm)(bacteria) | *S. aureus (+ )* (mm)(bacteria) | *C. albicaus* (mm) (fungi) |
|---|---|---|---|
| **[Eug/ ZnO: CuO]** | 17 | 28 | 20 |
| **Amoxicillin** | 10 | 12 | 12 |

Eugenol enhances the phospholipid bilayer of the bacterial cell membrane's non-specific permeability, which is the main antibacterial mechanism. The presence of OH on eugenol significantly reduces the activity of bacterial enzymes, further disrupts the bacterial membrane, and causes the release of internal components and cell death [27]. Additionally, it appears that the presence of metallic elements in composites improves the prevention of bacterial development by infiltrating the bacterial cell and killing it [28].

### Antioxidant Activity

The biological activity of [Eug/ ZnO: CuO] nanocomposite was tested by the DPPH method [29]. The results of the nano compound showed antioxidant activity against the DPPH free radical and provided a good scavenging percentage, and the $IC_{50}$ value of the synthetic compound was extracted, as shown in Table 4.

**Table 4. Scavenging (%) for nanocompound.**

| Nanocomposite | Synthesized Method | Scavenging % 6.25 µg\ml | 12.5 µg\ml | 25 µg\ml | 50 µg\ml | 100 µg\ml | Linear eq. | $R^2$ | $Ic_{50}$ |
|---|---|---|---|---|---|---|---|---|---|
| [Eug/ ZnO: CuO] | Sol-gel | 41.9 | 47.3 | 50.7 | 55.8 | 77.9 | y = 0.3611x + 40.729 | 0.9789 | 25.7 |
| Ascorbic acid | / | 51.4 | 57.4 | 61.1 | 64.7 | 93.2 | y = 0.4162x + 49.433 | 0.9572 | 1.4 |

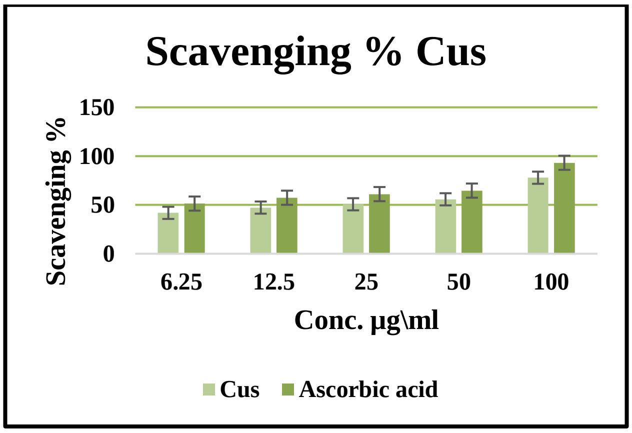


**Figure 10. Antioxidant activity of [Eug/ ZnO: CuO] nanocomposite**.

### Anticancer Activity

Eugenol has been shown to have several therapeutic benefits, including anticancer properties [7], which stop the spread of cancer by regulating the expression of many genes related to cell proliferation, angiogenesis, and death [30].

The cytotoxic effect of nano compound was examined with human breast cancer cells (MCF-7) using (MTT) assay method [31] from the tissue culture Laboratory in the Biotechnology Research Center Organization in Al-Neahrain University. The cytotoxic effect of [Eug/ ZnO: CuO] in the concentration range from 6.25-400 µg/ml on (MCF-7) cells line as shown in Table 5 reduction in cell viability in a dose-dependent pattern. Increasing the [Eug/ ZnO: CuO] concentration lowers cell viability. The decrease in (MCF-7) cell viability (%) was noted by 400 µg/ml (53.40 ±3.31) while the highest (MCF-7) cell viability at 6.25 µg/ml reached 95.99 ±0.96. The [Eug/ ZnO: CuO] exhibited significantly the most potent cytotoxic activity with an $IC_{50}$ value of 147.8 µg/ml on the (HdFn) normal cell line. However, an $IC_{50}$ of 133.5 µg/ml was obtained from the effect of [Eug/ ZnO: CuO] on (MCF-7) cell line as shown in Fig. 11. Through the values of $IC_{50}$, we conclude that the [Eug/ ZnO: CuO] nanocomposite needs a high concentration to kill (MCF-7) cell line and at the same time, its effect is little on normal cell (HdFn).

**Table 5. Cytotoxicity effect of [Eug/ ZnO: CuO] on (MCF-7) and (HdFn) cells.**

| [Eug/ ZnO: CuO] (µg/mL) | Viable cell count of (MCF-7) cell line Mean± SD. | Viable cell count of (HdFn) cell line Mean± SD. |
|---|---|---|
| **400.00** | 53.40 ±3.31 | 72.65 ±1.96 |
| **200.00** | 67.62 ±9.26 | 80.21 ±3.11 |
| **100.00** | 76.17 ±5.29 | 85.65 ±3.32 |
| **50.00** | 95.45 ±0.88 | 94.17 ±0.77 |
| **25.00** | 97.15 ±1.35 | 96.18 ±0.23 |
| **12.50** | 95.87 ±3.28 | 94.29 ±2.98 |
| **6.25** | 95.99 ±0.96 | 96.07 ±0.12 |

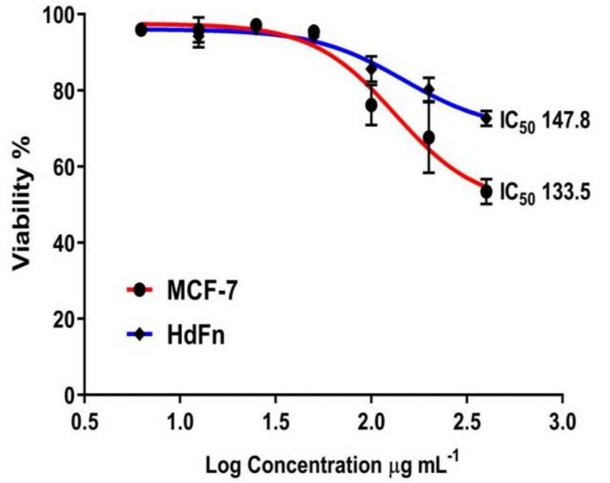


**Figure 11. Cytotoxicity effect of [Eug/ ZnO: CuO] on (MCF-7) and (HdFn) cells.**

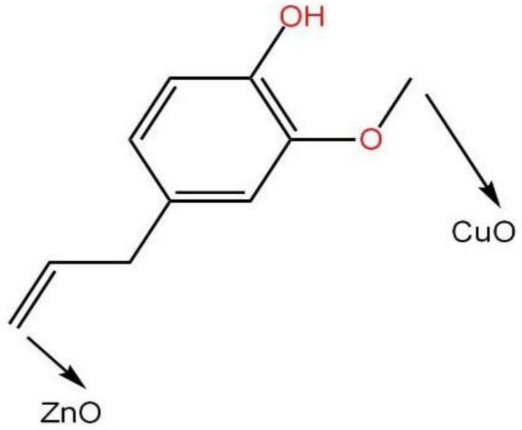


**Figure 12. Suggested structure of [Eug/ ZnO: CuO] nanocomposite.**

## Conclusion

The nanocomposite used to be synthesized with the aid of reacting eugenol with two metallic acetates [zinc acetate Zn $(CH_3CO_2)_2$ and copper acetate Cu $(CH_3CO_2)_2$] by way of the sol-gel method. The result of the measurements confirmed that the composite was synthesized as a nanocomposite, where the nanoparticle's mean diameter is in the nanoscale range. Also, different measurements had been made, such as (FT-IR, SEM, EDX, and XRD). The effectiveness of the [Eug/ ZnO: CuO] nanocomposite used to be examined on antibacterial and antifungal activity against two types of bacteria [*Escherichia Coli (-) (E. coli), Staphylococcus aureus (+) (S. aureus)*], and one type of fungi [*Candida albicaus (C. albicaus)*], confirmed a suitable response. The antioxidant activity of the [Eug/ ZnO: CuO] nanocomposite against the free radical showed a good scavenging percentage. In addition, the cytotoxicity effect of the [Eug/ ZnO: CuO] nanocomposite on breast cancer cells (MCF-7) was studied, and it showed acceptable results in killing the (MCF-7) cell line at high concentrations, whilst at the same time its effect is little on normal cells (HdFn). The research contributed to adding knowledge to the preparation of a new nanocomposite, as well as it includes a mixture of oxides on the eugenol matrix. The results of measurements for diagnosing the prepared nanocomposite were discussed according to approved scientific foundations, despite the absence of previously prepared nanocomposites close to it.

## Acknowledgment

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## Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

## Authors' Contribution Statement

F.A.A.: I did the conception of paper and acquisition and analysis of the data. B.I.A.: conceived the idea of the paper.

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# التخليق الأخضر للمتراكب النانوي: بناءً على [الأوجينول وأكاسيد المعادن], التشخيص والتطبيقات الطبية الحيوية

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**الخلاصة**

تم تفاعل الأوجينول (EUG) كمادة أساس مع خليط من أكاسيد المعادن (ZnO و CuO) التي تم تحضيرها من خلات المعدن، Zn $(CH_3CO_2)_2$ و $Cu (CH_3CO_2)_2$ كمواد أولية واستخدام الكلايكول إيثيلين (EG) كمذيب لتخليق المتراكب النانوي [ Eug/ ZnO: CuO] باستخدام طريقة Sol-Gel. تم تشخيص المتراكب النانوي المحضر باستخدام التقنيات (FT-IR, AFM, SEM, EDX, وXRD), حيث وجد أن متوسط قطر الجسيم يقع ضمن نطاق المقياس النانوي. ولوحظ أيضاً أن المادة النانوية المحضرة كانت على شكل قضبان ذات توزيع متجانس جيد. من أجل الوقوف على الخواص النانوية التي تم الحصول عليها، تم توظيف تلك الخواص من حيث البعد النانوي وخصائص الشكل، لدراسة فعالية المتراكب النانوي المحضر [Eug/ ZnO: CuO] كنشاط مضاد للميكروبات (مضاد للبكتيريا ومضاد للفطريات) ضد نوعين من البكتيريا *[ Escherichia Coli (-) (E. coli), and Staphylococcus aureus (+) (S. aureus)]*, ونوع واحد من الفطريات *[Candida albicaus (C. albicaus)]*, حيث أظهرت نتائج مقبولة. كما تم قياس فعالية المتراكب النانوي المحضر كمضاد للأكسدة ضد الجذور الحرة وأظهر نسبة كسح جيدة. بالإضافة إلى ذلك تمت دراسة التأثير السمي الخلوي للمتراكب النانوي [Eug/ ZnO: CuO] على خلايا سرطان الثدي (MCF-7)، وأظهر نتائج مقبولة في قتل الخط الخلوي (MCF-7) بتراكيز عالية.

**الكلمات المفتاحية:** الطب الحيوي, الأوجينول , التخليق الأخضر, اكاسيد المعادن, متراكب نانوي.