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Fabrication of Cr_2O_3 Nanoparticles Using Okra Plant Extract for Antimicrobial Activity

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ABSTRACT

Cr_2O_3 NPs were synthesized using Okra extract with two methods; the simple chemical method and sol-gel method. XRD analysis showed that the crystal size of Cr_2O_3 in a simple chemical method was (16.15) nm and with sol-gel method were 16.6 nm, 16.14 nm and 16 nm using annealing temperature of 200, 400 and 600 °C respectively. FE-SEM analysis showed that the nanoparticles of Cr_2O_3 NPs when using a simple chemical and the sol-gel methods, with particle sizes of (83.74-132.1) nm, (18.92-27.68) nm, (24.19-39.08) nm, and (12.04-15.83) nm that corresponding to a simple chemical method at 200 °C, sol-gel method at 200, 400 and 600 °C respectively. UV-Vis demonstrated that the band gap value of Cr_2O_3 NPs was 2.9 eV with a simple chemical method, while in a sol-gel method was (3.15, 3.3, 3.5) eV at different temperatures. The study showed an effective antibacterial activity of Cr_2O_3 NPs such was (12-14) mm of Gram's negative bacteria and (15-18) mm for Gram's positive bacteria in simple chemical method at (200) °C. The higher antibacterial activity of three different temperatures of sol-gel method was (20) mm for Gram's negative bacteria and (23) mm for Gram's positive bacteria at 200°C.

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تصنيع جسيمات Cr_2O_3 النانوية باستخدام مستخلص نبات البامية للنشاط المضاد للميكروبات

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قسم الفيزياء، كلية العلوم الجامعة، المستنصرية، بغداد، العراق

الكلمات المفتاحية:

جسيمات Cr_2O_3 النانوية
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فعالية مضادات البكتريا

الـــخُـــلاصـــة

تـم تصـنيع الجسـيمات النانويـه لأوكسـيد الكـروم الثلاثـي باسـتخدام مسـتخلص الباميـة بطريقتين. الطريقة الكيميائية البسيطة وطريقة *sol-gel* . أظهر تحليل حيود الأشعة السينيه *(XRD)* أن حجم بلـورة Cr_2O_3 بطريقة كيميائيـة بسيطة كـان (١٦.١٥) نـانومتر وبطريقة سول-جل كانت ١٦.٦ نانومتر و ١٦.١٤ نانومتر و ١٦ نانومتر باستخدام درجة حرارة التلدين

٢٠٠ و ٤٠٠ و ٦٠٠ درجة مئوية على التوالي. أظهر تحليل *FE-SEM* أن الجسيمات النانوية لـ *Cr2O3 NPs* عند استخدام طرق كيميائية بسيطة وطرق *sol-gel* ، بأحجام جسيمات تبلغ (٨٣.٧٤-١٣٢.١) نانومتر ، (١٨.٩٢-٢٧.٦٨) نانومتر ، (٢٤.١٩-٣٩.٠٨) نانومتر ، و (١٢.٠٤-١٥.٨٣) نانومتر المقابلة لطريقة كيميائية بسيطة عند ٢٠٠ درجة مئوية ، طريقة سول جل عند ٢٠٠ و ٤٠٠ و ٦٠٠ درجة مئوية على التوالي. أظهر *UV-Vis* أن قيمة فجوة النطاق لـ *Cr2O3 NPs* كانت ٢.٩ فولتًا باستخدام طريقة كيميائية بسيطة ، بينما في طريقة *sol-gel* كانت (٣.١٥ ، ٣.٣ ، ٣.٥) فولت عند درجات حرارة مختلفة. أظهرت الدراسة فعالية فعالة مضادة للبكتيريا لـ *Cr2O3 NPs* مثل (١٢-١٤) ملم من البكتيريا سالبة الجرام و (١٥-١٨) ملم للبكتيريا الموجبة للجرام بطريقة كيميائية بسيطة عند (٢٠٠) درجة مئوية. أعلى نشاط مضاد للبكتيريا لثلاث درجات حرارة مختلفة لطريقة سول-جل كان (٢٠) ملم للبكتيريا سالبة الجرام و (٢٣) ملم للبكتيريا الموجبة للجرام عند ٢٠٠ درجة مئوية.

1. Introduction

Studying of nanoparticles has received an increasing attention due to the new properties that the material may exhibit when the particle size is reduced [1]. Nanotechnology is one of the most active research areas in modern materials science [2]. Nanoparticles, especially transition-metal oxides play a significant role in many areas of chemistry, physics and materials science [3, 4].Chromium (III) oxide (Cr_2O_3) is the most stable of the several chromium oxides throughout a wide range of temperatures and pressures [5]. The (Cr_2O_3) is technologically important because of its several applications, which include green dyes, catalysts, UV protection coatings, and visible light transmittance. (Cr_2O_3) is the most powerful oxide with a variety of appealing properties, including a low coefficient of friction, high wear resistance, good optical and thermoelectric properties, and a high melting point (2435°C). It has also a high melting point (2435 °C) and high temperature oxidation resistance. Depending on the synthesis circumstances, the (Cr_2O_3) films can display either p-type or n-type semiconductor behavior, despite their intrinsic insulating nature. It is a favored material for developing a wide range of industrial applications due to the combination of all of these features [6]. In this context, the (Cr_2O_3)NPs are of great among nanoparticles based on various metal oxides due to their unique physical and chemical properties such as wide band gap (~3.4 eV), high melting temperature, and increased stability [7]. The poisonous effects of (Cr_2O_3) nanoparticles can be reduced by either coating or functionalization of their surfaces with biogenic materials. One of the most promising ways of achieving this is surface coating of the (Cr_2O_3) is with plants' biogenic phytomolecules .The synthesis of nanoparticles using plants as a precursor has attracted much attention recently. As an alternative to conventional chemical and physical methods, the green synthesis of nanoparticles using biological sources (plants) is an economical, robust, ecofriendly, and easily scalable technique [8]. Biomedical reducing agents are avaiable at diff erent concentrations in diff erent types of leaf extracts, so the leaf extract composition has a great eff ect on the nanoparticle synthesis [9,10]. Metal Oxide NPs' attributes are with determined Size, form, surface area, crystallinity, and stability are all controlled by the synthesis procedure and circumstances. Chemical processes such as sol-gel synthesis, hydrothermal process, and chemical vapor deposition, as well as physical methods such as ball milling, electro spraying, or sputtering, can all be used to make metal oxide NPs [11]. Wet chemical techniques are chosen because they allow for better control of size, content, and structure. For ages, chromium (III) oxide has been used as a pigment in paints, inks, and glassware [12]. The Cr_2O_3 is technologically important due to its numerous applications, including green pigment, catalysts, coating materials for UV protection, and visible light transmittance [13]. It is the hardest oxide that also exhibits much attractive functionality among this lower coefficient of friction, high wear, and corrosion resistance, and good optical and adiabatic characteristics are key properties. It also exhibits a high melting point (2435 ºC) and high-temperature oxidation resistance [14, 15]. Cellulose is the most sustainable and renewable resource, which has attracted much attention in the last decades and stimulated researchers to develop cellulose-based materials with novel functions. It shows high strength, low density, high crystallinity along with biodegradability and biocompatibility [16-20]. In this work, Cr_2O_3NPs were prepared from (Okra) extract with chromium (III) chloride ($CrCl_3$) by a simple chemical method and a sol-gel method. The structural and optical properties of Cr_2O_3 NPs in two methods were determined. High-resolution X-ray diffraction (XRD) was used to assess the crystalline quality of the samples produced, and scanning electron microscopy (SEM) was used to investigate the surface morphology. UV–vis spectrophotometer was used to investigate their optical properties. The aim of this study is to prepared chromium oxide Cr_2O_3 nanoparticles from cheap starting materials, in a simple and easy method (simple chemical method and sol-gel method) and evaluate their structure, shape, size, and surface morphological.

2. Materials and Methods.

2.1 Materials

All the chemicals materials were of high purity without further purification or distillation: These include [$CrCl_3.6H_2O$] (99.5%, Sigma-Aldrich), sodium hydroxide (NaOH) (99%, Fluka), Okra extract; were collected from local market.

2.2 Preparation of plant extract

In a (simple chemical and sol-gel) methods, small parts of the corn plant were purified to remove impurities, cut up and left in the sun for two days. The Okra was then ground to a fine powder with a solid metal electric mixer. The plant extracts were produced from a mixture of 5 g of powder dissolved in 100 ml deionized water. The solution was then heated at 70 ° C for 30 min on a magnetic stirrer. The resulting aqueous solution was cooled at a room temperature and purified using Whitman's filtration.

2.2.1 Preparation of Chromium oxide NPs using simple chemical method

To produced Cr_2O_3 NPs (50 mL, 1 M) chromium chloride hexahydrate ($CrCl_3.6H_2O$) was prepared into 100 mL Okra extract. This prepared solution was then placed on a magnetic stirrer at 70 ° C for 30 min. The altered interaction formed chromium NPs.The solution was left to cool at a room temperature. 25 mL of Cr_2O_3 was placed in a ceramic oven at 200 ° C for two hours to obtain a nano-base.

2.2.2 Preparation of Chromium oxide NPs using Sol-Gel method

5 grams of Okra powder in 100 ml of distilled water and then put the solution on magnetic stirrer at 70°C, 13 grams of $CrCl_3$ was taken with 50 ml of distilled water for 10 minutes and then filtered , a centrifuge was used at 15,000 cycles for 10 minutes, $CrCl_3$ is added over the Okra extract for half an hour on the magnetic stirrer, and then it was placed to turn it into a gel After that, and place on the magnetic stirrer. Next several drops of sodium hydroxide were add to the solution until it attained a pH = 7. The temperature was increased to 90°C and left to turn into liquid (Gel). Thus it was allowed to stay for one and half an hour after which the temperature was increased to (120°C) when it left to become dry gel (Xerogel). , After

that it lifted and left to cool down. The powder was collected and placed in a container of porcelain and inserted into the oven 25 ml of the solution is taken and placed in a ceramic eyelid and placed at 200°C to two hr. After turning off the oven, the sample wasleft inside for 24 hours to obtain a nano powder.

2.3 Characterization of Chromium oxide NPs in simple chemical method and in a Sol-Gel method

XRD analysis was determined using JCPDS (Joint Committee for Powder Standards) with (CuKα) irradiation (1.5418 Au) as source. The samples of chromium oxide samples were examined by XRD measurements in an advanced nanotechnology laboratory in Iran. The morphology of butterflies' molecules of chromium oxide was analyzed by SEM by (Tescan Mira3-Czech) in Iran-Kashan. Liquid solution UV-Vis transmission spectra of NPs were constructed with a dual-beam spectrophotometer (UV-1800, Shimadzu) for a range of (200 to 900) nm.

1. Results and Discussion

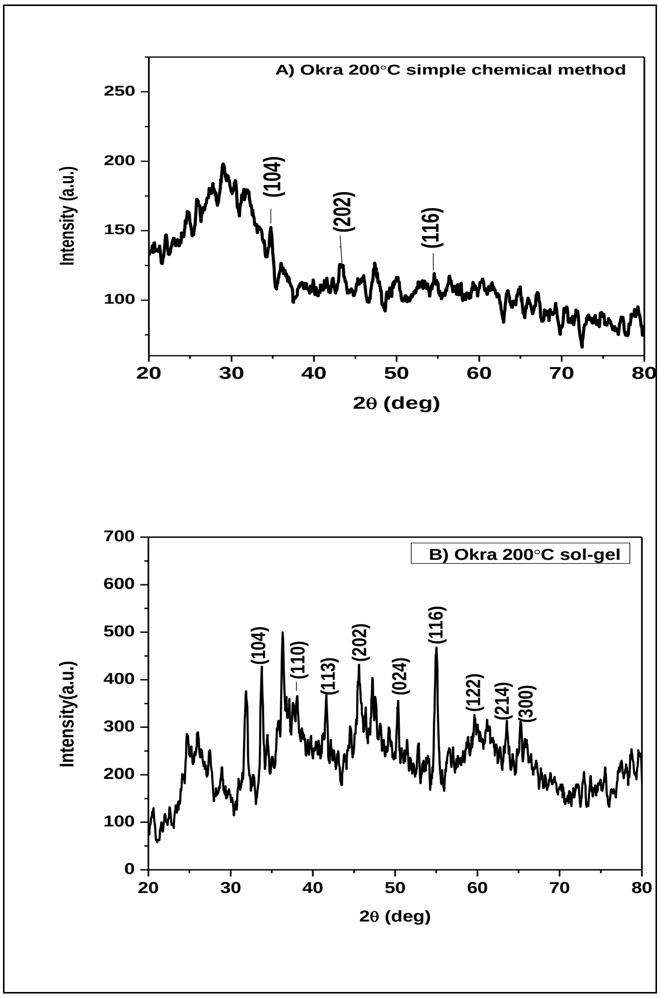
3.1 XRD analysis

Figure 1 explains the main bands which were parallel to chromium oxide which observe with annealing tempreture of 200°C in the simple chemical method ,the diffraction peaks at 2θ were 34.76◦ , 43.81◦ and 54.80◦ that corresponds to the (104),(202) and (116) planes of Cr_2O_3 and the peaks 33.71◦, 38.11◦, 41.66◦, 45.56◦, 50.33◦, 54.97◦, 59.62◦, 63.65◦, and 65.54◦ corresponds to the (104),(110),(113),(202),(024),(116),(122),(214) and (300) which are the preferred orientation of Cr_2O_3 NPs at 200°C in a sol-gel method, the diffraction peaks at 2θ with 24◦,33.82◦,38◦,41.62◦, 45.52◦,50.3◦,54.96◦,63.58◦ and 65.22◦ the peaks (012),(104),(110),(113),(202),(024),(116),(214) and (300) which are the preferred orientation of Cr_2O_3 NPs at 400°C JCPDS card No. (96-210-4123) In the sol-gel technique, the diffraction peaks at 2 with 22.48, 33.36, 37.68, 41.76, 44.5, 50.44, 54.61, 63.51, and 65.21 improved in 600 °C (JCPDS Card No. 98-004-5808), respectively. The latter corresponds to the Cr2O3 planes (012), (104), (110), (113), (202), (024), (116), (214), and (300). All of the peaks improved after annealing at 600°C.

Table 1.presents XRD results of $\mathbf{Cr_2O_3}$ NPs using okra extract. The crystallite sizes (D) were estimated by the following Scherrer's Equation (1).

$$D(nm) = \frac{K\lambda}{\beta \text{Cos}\theta} \dots\dots (1)$$

Where k stands for form factor (0.9), wavelength (0.15418), (CuK), full width at half maximum (FWHM), and diffraction angle.



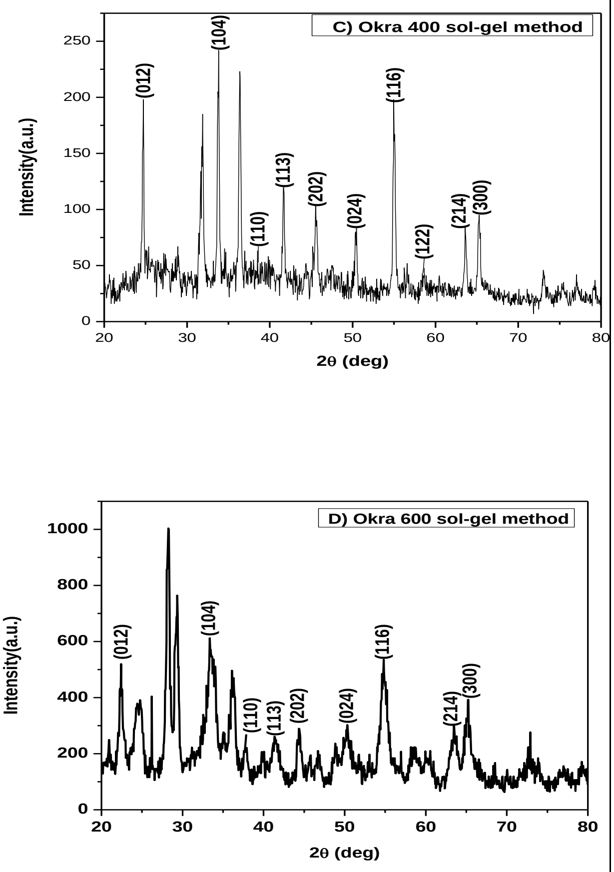


Figure 1. XRD pattern of Cr_2O_3 NPs using (Okra) extract by the simple chemical method at 200°C,and by the sol-gel method at (200,400,600) °C.

Table 1. XRD results of Cr_2O_3 NPs by the (simple chemical method at 200°C and sol-gel method at (200,400,600) °C using (Okra) extract.

Method	2θ (deg.)	hkl	2Ѳ JCPDS	Crystallite size D
Simple chemical at 200°C	34.76	(104)	33.2	16.15
	43.81	(202)	45.5	38.1
	54.80	(116)	55	52.7
Sol-gel at 200 °C	33.71	(104)	33.2	16.6
	38.11	(110)	37.55	13.2
	41.66	(113)	42	77
	45.56	(202)	45.5	38
	50.33	(024)	50	93
	54.97	(116)	55	53
	59.62	(122)	59.21	20.2
	63.65	(214)	64.1	64.2
	65.54	(300)	66	58.1
Sol-gel at 400 °C	24	(012)	23.1	20.8
	33.82	(104)	33.2	16.14
	38	(110)	37.55	13
	41.62	(113)	42	77.8
	45.52	(202)	45.5	38.3
	50.3	(024)	50	93
	54.96	(116)	55	53
	63.58	(214)	64.1	64.4
	65.22	(300)	66	57.2
Sol-gel at 600 °C	22.48	(012)	23.1	20.7
	33.36	(104)	33.2	16
	37.68	(110)	37.55	12.9
	41.76	(113)	42	78
	44.5	(202)	45.5	38.4
	50.44	(024)	50	94
	54.61	(116)	55	52.7
	63.51	(214)	64.1	64
	65.21	(300)	66	58.2

3.3 (SEM) analysis.

A crystallinity, particle size, surface, were all examined using SEM. Figure 2 shows a SEM bright field image from Cr2O3 NPs. The presence of spherical forms in Cr2O3 is clearly demonstrated. In the sol-gel technique, increasing the annealing temperature (200C) improves crystallinity. Figure 2 illustrate the SEM images of chromium oxide NPs using (Okra) extract in a simple chemical and sol-gel methods. The nanoparticles of Cr2O3 NPs are spherical or semispherical in shape, with particle sizes of (83.74-132.1) nm, (18.92-27.68) nm, (24.19-39.08) nm, and (12.04-15.83) nm, respectively, of a Simple chemical technique at 200°C, sol-gel method at 200°C, sol-gel method at 400°C, and sol.

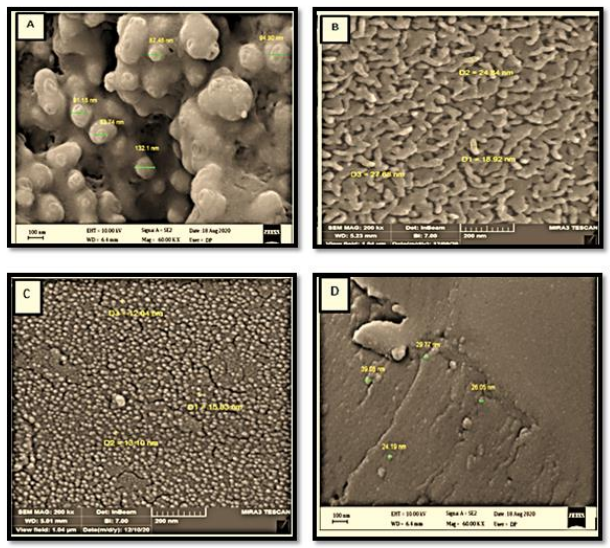


Figure 2. (A-D) The shape and average size of Cr_2O_3 NPs using Okra extract in scale (100-200) nm A)the Simple chemical method at 200°C , B)the sol-gel method at 200°C , C)the sol-gel method at 400°C , and D) the sol-gel method at 600 °C.

3.5 Optical properties of Cr_2O_3 NPs

Figure 3 explains optical transmittance spectrum of (Cr_2O_3) NPs as synthesized via the chemical method. It shows the strong absorption of (Cr_2O_3) spectral range from (200-900) nm. Effect of particle volume on the transmittance value can be observed.

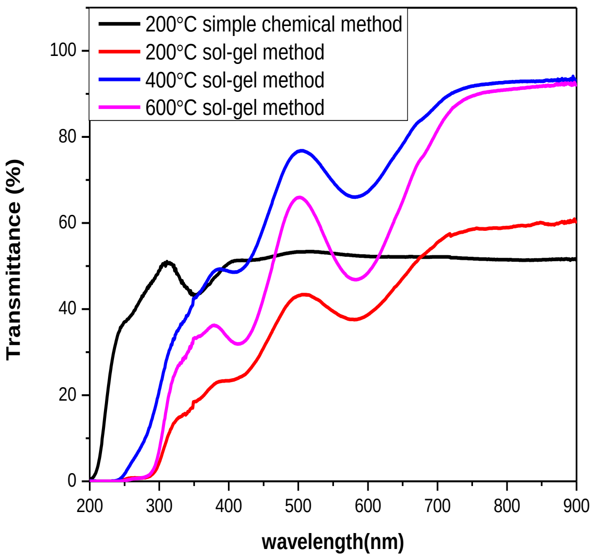
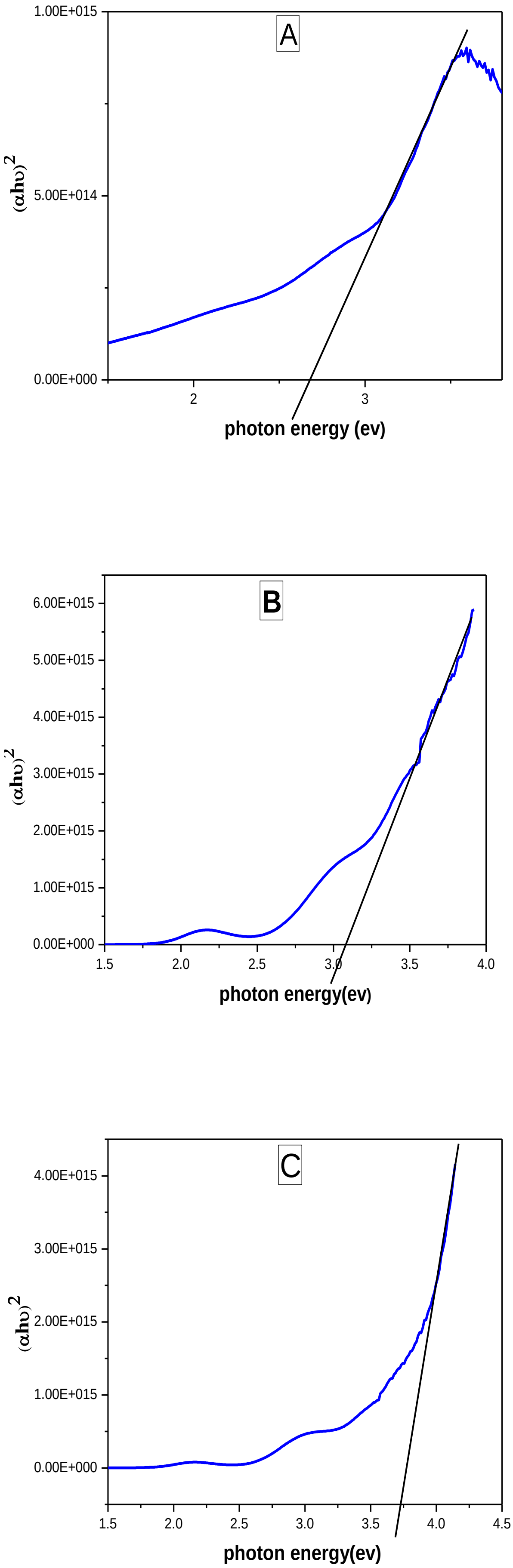


Figure 3. The Transmittance spectra of Cr_2O_3 nanoparticle for four samples .

Figure 4 shows the energy gap (Eg) of (Cr_2O_3) NPs prepared with Okra extract which calculated by graphing the square of $(h\nu)^2$ against the photon's energy ($h\nu$). The energy gap value was obtained by extrapolating a straight line to $(h\nu)^2$. The energy band (for example, the crystal structure of the film) and the arrangement and distribution of the atoms in the crystal lattice all influence the gap.



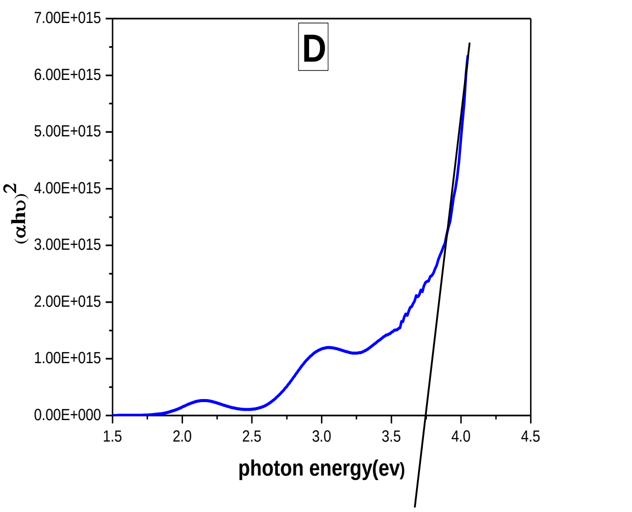


Figure 4. Plot of Cr_2O_3 nanoparticles (h)2 vs. photon energy (h). for simple chemical method at A)200 °C , the sol-gel method at B)200, C)400,and D)600)°C.

Table 2. The Particle size, crystallite size and energy band gap of chromium oxide NPs in two methods.

Plant extract	Method		Particle size D (nm)	Crystallite size (nm)	Energy gap (eV)
Okra	Simple chemical	200 °C	83.74-132.1	16.15	2.9
	Sol-gel	200 °C	18.92-27.68	16.6	3.15
		400 °C	24.19-39.08	16.14	3.3
		600 °C	12.04-15.83	16	3.5

Antibacterial Activity of Cr_2O_3 NPs

The antibacterial activity of chromium (NPs) that prepared by the simple chemical and sol-gel methods using (Okra) extract was been studied. The efficacy of anti-bacterial activity in terms of inhibition zones (mm) was been measured against two stander bacterial Gram-positive bacteria (Staphlococcus aureus, Staphlococcus epidermidis) and Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae) were isolated, as indicated in figure 5 and table3. The experiment was carried out in sterile 90 mm diameter petri plates with sterile nutrition agar medium employing agar well diffusion procedures. The PH was adjusted to 6.8 at 25 °C, and the components were combined and heated for about 15 minutes at 121 °C to ensure that they were thoroughly blended before being placed in petri dishes and covered until the solution solidified. The Cr_2O_3 NPs were then dissolved in a dimethyl Sulphoxide solution containing 40 mg/mL. (DMSO). As soon as the germs were ready, they were swabbed across the surface of the petri plates. Wells of 8 mm diameter were made in the bacterial and fungal medium of each plate using a sterile gel puncher / crok borer. There are 40 liters of Cr_2O_3 NPs in each well, as well as a DMSO control. Bacteria and fungus were added to the well at the required concentration. The inhibitory zone diameter was measured after the test samples were incubated at 25 °C for 24 hours. The antibacterial activity of Chromium oxide NPs produced using maize extracts utilizing a simple chemical approach (oxidation and reduction processes) was investigated[21-22]. The efficacy of anti-bacterial activity in terms inhibition of zones (mm) was measured against four stander \ gram-positive and gram negative bacterial isolates. Biosynthesis of the NPs showed a good inhibition efficacy, but biosynthesis of Chromium oxide NPs at 200°c in the sol-gel method was more effective than Chromium oxide synthesized at 200°C through a simple chemical method[23,24].

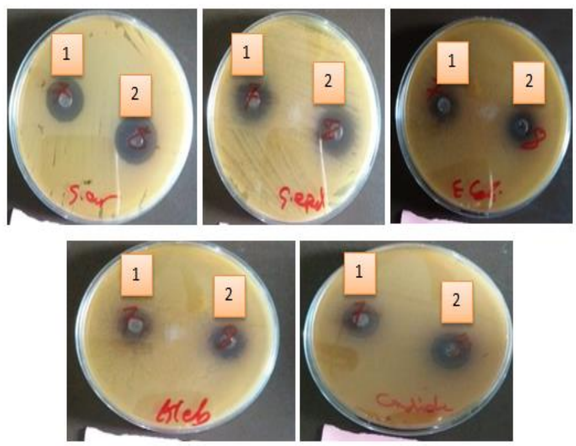


Figure 5. The antibacterial activity of Cr_2O_3 NPs synthesized by Okra extracts on different

Gram- negative and Gram positive bacteria. (1) Simple chemical method and (2) sol-gel method

Isolated bacteria	Inhibition zone of Cr_2O_3 NPs using simple chemical method	Inhibition zone of Cr_2O_3 NPs using sol-gel method
Staphylococcus aureus +	15	21
Staphylococcus epidermidies +	18	21
Escherichia coil -	*12*	*16*
Klebsiella up -	14	14
Candida albicons	*15*	*17*

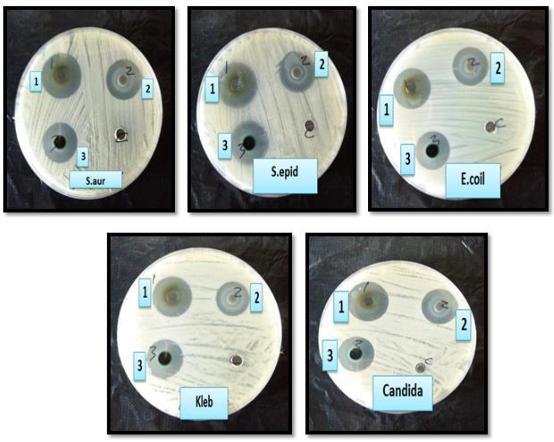


Figure 6. The antibacterial activity of Cr_2O_3 NPs synthesized by plant extracts on different Gram- negative and Gram positive bacteria.(1) the sol-gel method at 200 °C , (2) the sol-gel method at 400 °C and (3) the sol-gel method at 600 °C

Table 4. The antibacterial activity of the Chromium oxide nanoparticles synthesis by plant extracts against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*.

	Inhibition zone		
	Cr_2O_3 nanoparticles synthesized by		
Isolated bacteria	*Okra extract (simple chemical method)* 200 ° C	*Okra extract (sol-gel method)* 400 ° C	*Okra extract (sol-gel method)* 600 ° C
Staphylococcus aureus +	*23*	*21*	*20*
Staphylococcus epidermidies +	*22*	*20*	*21*
Escherichia coil -	*20*	*19*	*16*
Klebsiella up -	20	17	*19*
Candida albicons	*23*	*20*	*19*

3. Conclusions

In this work a chromium oxide nanoparticles were prepared using (Okra) extract using the simple chemical and sol-gel methods. Plant extracts could successfully convert CrCl3.6H2O into chromium oxide nanoparticle. Plant extracts also possess reducing, and anti-agglomeration effects. The results with the sol-gel method were better than the results with the simple chemical method. This was due to an increase in purity and change of phases, a decrease in the size of particles, and finally an increase in the energy gap. The results obtained by a simple chemical method were less purity than the sol-gel method.

The results of the present study showed that chromium nanoparticles synthesized from (Okra) extracts displayed an effective antibacterial action on the test isolates, as indicated by the diameter of the inhibition zone. Comparing the simple chemical method and the sol-gel method with 200° C, the sol-gel method was superior for both gram-negative bacteria and gram-positive bacteria.These nanostructures used to study the antibacterial and antifungal activity. The green synthesis of the nanostructure using plants materials as reducing and capping agent having advantages like ease in availability, very simple, cost low, eco-friendly with which the process can be scaled up economic viability with high-quality products of different nanostructure.

4. Novelty:

The excellent results obtained in this work encourage us to offer some suggestions for the development of future studies.

1- Our findings could be targeted for promising and potential applications including drug formulation and biomedical applications in the future.

2- Several novel green approaches methods for synthesizing NPs using different natural sources such as algae, bacteria, and fungi extracts and evaluation of their catalytic activity.

3- Synthesizing nanostructures by plants extract using pulsed laser ablation method.

4- Preparing oxide materials nanostructures such as (Chromium Oxide) using plant extracts, and apply the products in different applications like (solar cells, detectors, and sensors).

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