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Antibacterial activity of Cr_2O_3 nanoparticles against E.coli; Reduction of chromate ions by *Arachis hypogaea leaves*

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ABSTRACT

Chromium oxide nanoparticles (NPs) were rapidly synthesized by reduction of potassium dichromate solution with Arachis hypogaea leaf extract containing reducing sugars which act as reducing agent. The results indicated that the aldehyde groups present in the plant extract played an important role in the formation of Cr_2O_3 nanoparticles. The purification process of the Cr_2O_3 product does not require expensive methods, since a solid product is obtained from a reaction in liquid phase. The antibacterial effect of Cr_2O_3 nanoparticles against Escherichia coli was investigated as a model for Gram-negative bacteria. Bacteriological tests were performed in Potato Dextrose Agar (PDA) medium on solid agar plates and in liquid systems supplemented with different concentrations of nanosized Cr_2O_3 particles. These particles were shown to be an effective bactericide. The resulting Cr_2O_3 nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), UV-VIS absorption and Fourier-transform infrared (FTIR) spectroscopy.

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Keywords: *Arachis hypogaea* L., Potassium dichromate solution, Cr_2O_3nanoparticles, *E.coli*

INTRODUCTION

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology because biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional physical and chemical methods.

Transition metal oxide nanoparticles represent a broad class of materials that have been researched extensively due to their interesting catalytic, electronic and magnetic properties. Chromium oxides have attracted much attention recently because of their importance both in science and technology. Special attention has been focused on the formation and properties of Chromia (Cr_2O_3), which is important in specific applied applications such as in high-temperature resistant materials[1], corrosive resistant materials[2], liquid crystal displays[3, 4], green pigment [5], catalysts[6,7] and so on. It is well known that intrinsic properties of inorganic materials are mainly determined by their composition, structure, crystallinity, size and morphology; great efforts have been devoted to the investigation of different chromium oxide materials synthesis [8-10].

Various techniques have been developed to synthesize Cr_2O_3 nanoparticles such as precipitation [11], precipitation-gelation [12-14], sol gel [15-17], mechanochemical reaction, oxidation of chromium in oxygen [18] and sonochemical methods [19]. Among all these methods as mentioned, chemical reduction in aqueous solvents

exhibits the greatest feasibility to be extended to further applications in terms of its simplicity and low cost. The green chemical method employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures and physical methods.

The green synthesis techniques are generally synthetic routes that utilize relatively non-toxic chemicals to synthesize nanomaterials and include the use of non-toxic solvents such as water, biological extracts, biological systems and microwave assisted synthesis. The *Arachis hypogaea* leaves posses biomolecules such as carbohydrates, amino acids and vitamins[20], which could be used as reducing agent to react with chromium ions and as scaffolds to direct the formation of Cr_2O_3 NPs in solution. To the best of our knowledge, the use of plant extracts at room temperature for the green synthesis of Cr_2O_3 nanoparticles has not been reported.

Khatoon et al [21] reported the internalization of chromium oxide nanoparticles in *Escherichia coli* cells was evaluated by flow cytometry using light scattering method. El-ajaily et al [22] reported the antibacterial activity of Cr (VI) and Cr (III) complexes against *P. aeruginosa* bacteria. Singh et al [23] reported viability of an environmentally relevant bacterium, *E. coli* exposed to varying concentrations of Chromium oxide nanoparticles was evaluated PMA (Propidium mono-azide)assisted Q-PCR. Vadde Ravinder et al [24] reported the antibacterial effects of chromium (III) complexes using single representative strains of *E. coli* and *Bacillus subtilis*. Although only a few studies have reported the antibacterial properties of chromium (III) complexes have a significant promise as bactericidal agent.

MATERIALS AND METHODS

Materials

Potassium dichromate was purchased from Merck without further purification. *E. coli* (ATCC 8739) strain was purchased from Institute of microbial Technology (Chandigarh, India). The components of the Luria–Bertani (LB) medium used in growing and maintaining the bacterial cultures were supplied by Projen Laboratories (Chennai, India).

Preparation of *Arachis hypogaea* leaf extract

About 20g of freshly, taxonomically authenticated healthy leaves of *Arachis hypogaea* L. were collected, washed thoroughly with double distilled water, cut in to fine pieces and boiled with 100mL double distilled water in Erlenmeyer flask for 8-10 min. The extract was cooled to room temperature and filtered through Whattman filter paper (no.42).

Preparation of Potassium dichromate solution

14.5g of potassium dichromate was dissolved in 50 mL deionized water and stirred for 15 min. An orange solution of potassium dichromate solution was obtained.

Preparation of Cr_2O_3 Nanoparticles

In a typical experiment 10 ml of potassium dichromate solution was mixed with 10 ml of the *Arachis hypogaea* leaf extract in a beaker. After 10 minutes, the colour of the solution changed from orange to green indicating the formation of chromium oxide nanoparticles .The solution was kept at room temperature for evaporation of aqueous phase.

Antibacterial assay

The Cr_2O_3 nanoparticles synthesized using *Arachis hypogaea* was tested for antibacterial activity by agar well-diffusion method against *Escherichia coli*. The pure bacterial culture was subcultured on nutrient agar and Potato Dextrose Agar (PDA) respectively. Wells of 10 mm diameter were made on nutrient agar and PDA plates using gel puncture. The strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl, 30 µl and 40 µl) was poured onto each well on the plates. After incubation at 37°C for 24 hours, the different level of zone of inhibition of bacteria was measured.

Characterization

Characterization of the as-prepared Cr_2O_3 nanoparticles was carried out by different techniques. UV-VIS spectra were measured using a TU-1901 model UV- VIS double beam spectrophotometer (Beijing Purkinje General

Instrument Co., Lt, China). FTIR spectra were performed and recorded with a Fourier-transform infrared spectrophotometer (Nicolet 870) between 4000cm^{-1} and 400cm^{-1}, with a resolution of 4cm^{-1}. The morphological properties of the Cr_2O_3 nanoparticles were examined by Scanning Electron Microscopy (SEM), using a LEO 1455 VP equipped with energy dispersive X-Ray Diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffracto meter Using CuKα radiation (λ= 1.5406 Å).

RESULTS AND DISCUSSION

The addition of potassium dichromate solution to the plant extract containing a mild reducing agent, such as glucose, causes the reduction of orange dichromate (VI) ions to green chromium (III) ions. The reduction of Cr^{6+} to Cr^{3+} by reducing sugars results in the formation of Cr_2O_3 nanoparticles. In turn the aldehyde is oxidized to the corresponding carboxylic acid.

The chemical reaction which occurs

$$3RCHO + Cr_2O_7^{2-} + 8H^+ \longrightarrow 3RCOOH + 2Cr^{3+} + 4H_2O \quad (1)$$

The electron-half-equation for the reduction of dichromate (VI) ions is:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \longrightarrow 2Cr^{3+} + 7H_2O \quad (2)$$

Combining that with the half-equation for the oxidation of an aldehyde under aqueous conditions:

$$RCHO + H_2O \longrightarrow RCOOH + 2H^+ + 2e^- \quad (3)$$

In fact, the reduction of Cr^{6+} to Cr^{3+} plays a main role in this process and then Cr_2O_3 is generated through the following reactions.

$$2Cr^{3+} + 3H_2O \longrightarrow Cr_2O_3 + 6H^+ \quad (4)$$

Therefore, we took advantage of the ready reactivity of solution with reducing sugars to innovate a facile method for the synthesis of Cr_2O_3 nanoparticles.

The reaction between metal ions and the leaf extracts was confirmed by UV-Visible spectra of Cr_2O_3 nanoparticles in aqueous solution are shown in Figure 1. The peak at 458nm is due to inter band transition of core electrons of chromium and chromium oxide [25]

The possible biomolecules responsible for the stabilization of Cr_2O_3 nanoparticles were identified by FT-IR spectroscopic studies. The representative absorption peaks in FT-IR spectra of nanoparticles located mainly at 3411cm^{-1}, 1311cm^{-1}, 947cm^{1} and 564cm^{-1} in the region 400cm^{-1}-4000cm^{-1} shown in Figure 2. The peak at 3411cm^{-1} is the characteristic band of hydrogen bonded OH groups present in the aqueous phase. The peaks at 1311cm^{-1} indicate the presence of (-COO-) carboxylate ions, responsible for stabilizing the Cr_2O_3 nanoparticles. The peak at 947cm^{-1} and 564cm^{-1} indicates that Cr=O and Cr-O vibration of Cr_2O_3 nanoparticles [26].

The X- ray diffraction patterns obtained for the Cr_2O_3 nanoparticles synthesized using *Arachis hypogaea* L. extract is shown in Figure 3. The XRD spectrum contains three peaks that are clearly distinguishable. All of them can be perfectly indexed to crystalline Cr_2O_3 not only in peak position, but also in their relative intensity. The peaks with 2θ values of 27.3°, 36.8°, 49.7° and 64.7° are corresponds to the crystal planes of (012), (110),(024) and (214) of crystalline Cr_2O_3 respectively. The crystallite sizes can be estimated using Scherer's formula $D = K\lambda / \beta \cos\theta$ where the constant K is taken to be 0.94, λ is the wavelength of X-ray and β and θ are the half width of the peak and half of the Bragg angle respectively. Using the equation, the crystallite sizes found to be in the range of 60-80nm.

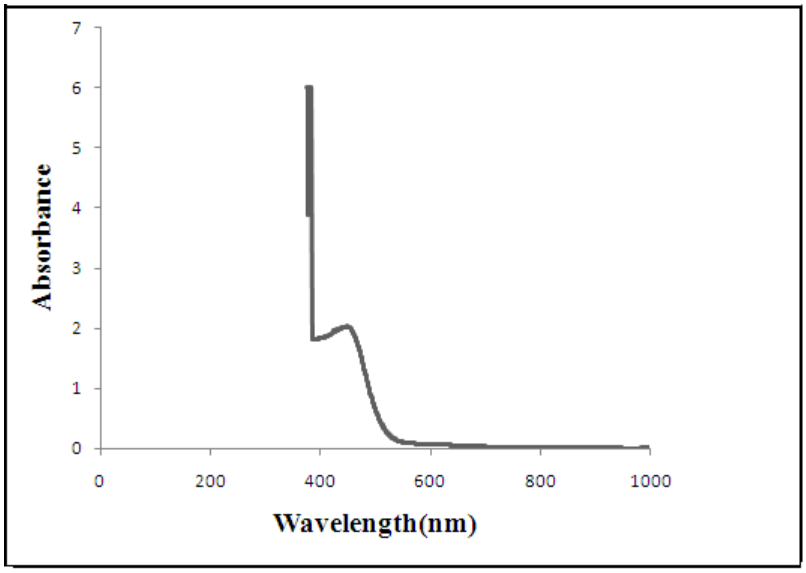

Fig. (1). UV-Vis absorption spectrum of Cr_2O_3 nanoparticles in aqueous solution

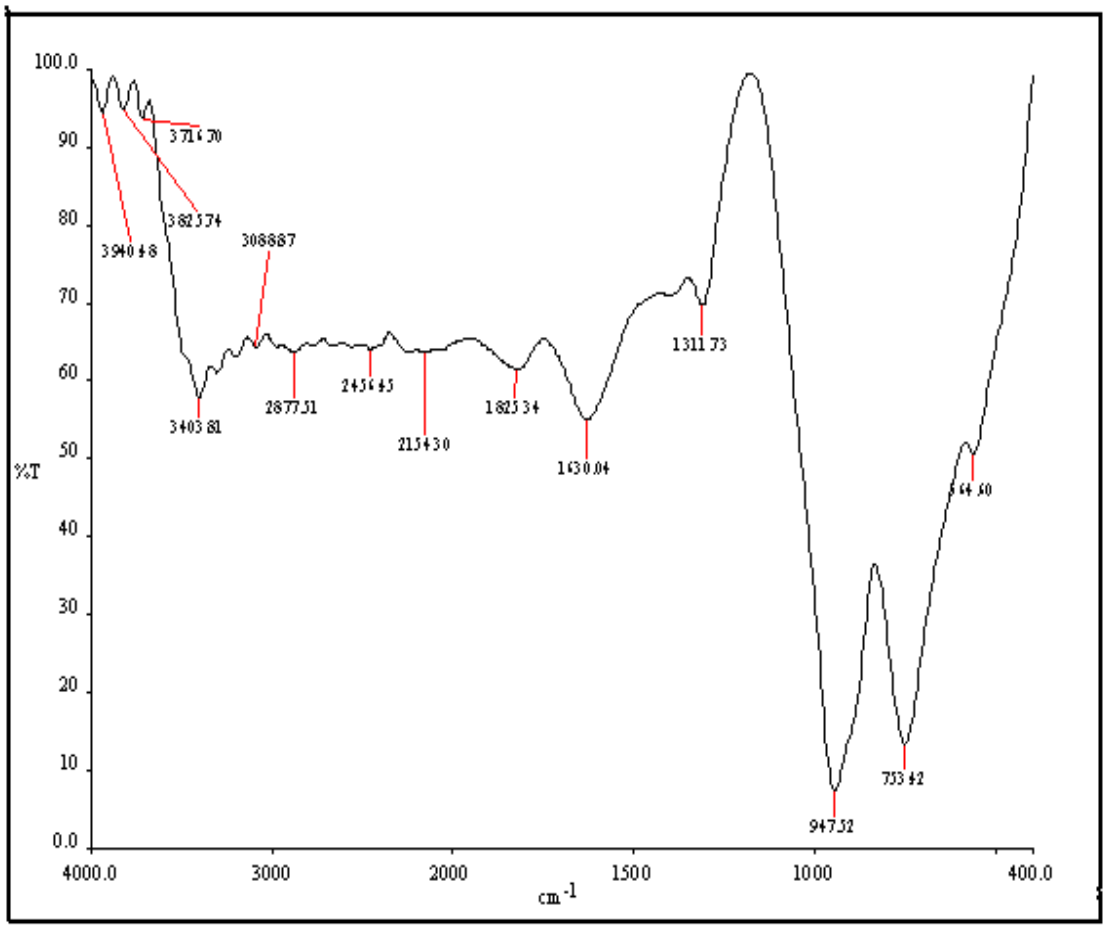

Fig.(2). FT-IR spectra of Cr_2O_3nanoparticles synthesized using *Arachis hypogaea* leaf extract

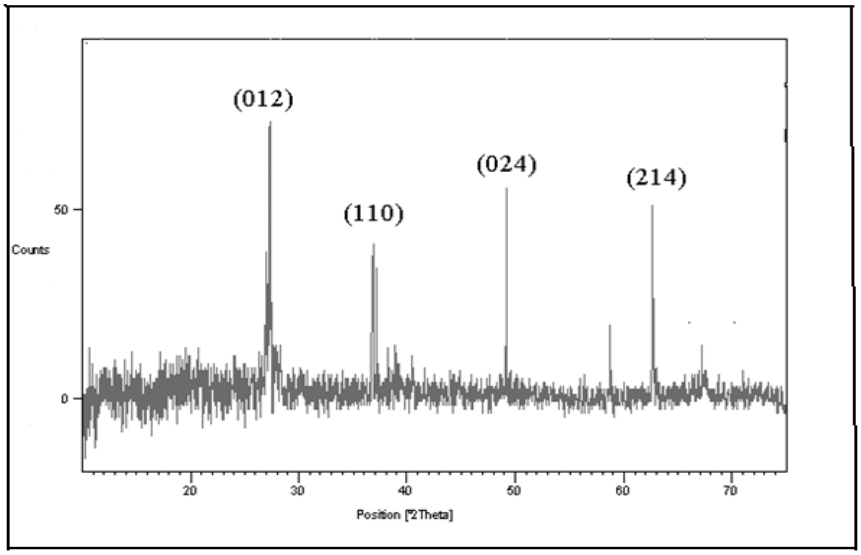

Fig. (3). XRD pattern of Cr_2O_3 nanoparticles synthesized using *Arachis hypogaea* leaf extract

The morphological studies of synthesized Cr_2O_3 nanoparticles were analyzed by scanning electron microscopy is shown in Fig 4(a & b). It was identified that shapes of the Cr_2O_3 nanoparticles appeared like hexagonal and cubic with rough surfaces. All the nanoparticles were well separated and no agglomeration was noticed.

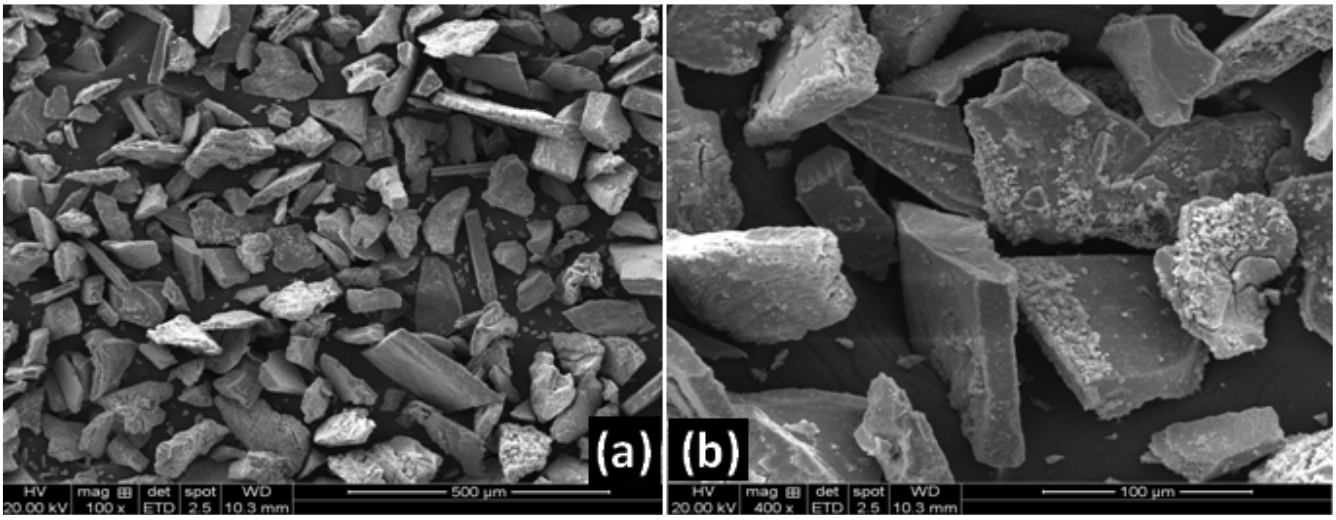

Fig 4 (a & b). SEM micrographs of Cr_2O_3 nanoparticles synthesized using *Arachis hypogaea* leaf extract at different magnification level.

The antibacterial activity of *Arachis hypogaea* mediated Cr_2O_3 nanoparticles was performed against *Escherichia coli* using agar well diffusion method. The mean of three replicates of zone of inhibition (mm) around well with *Arachis hypogaea* mediated Cr_2O_3 nanoparticles is presented in the Table 1.

Table 1. Zone of inhibition (mm) of *Arachis hypogaea* mediated Cr_2O_3 nanoparticles (µl)

Test organism	Concentration of nanoparticles (µl)			
	10 µl	20 µl	30 µl	40 µl
Escherichia coli	2.2	2.6	3.0	3.2

The Figure 5 shows the zone of inhibition of bacterial growth on agar plates as a function of the different concentration of Cr_2O_3 nanoparticles when gradually declined when the concentration of nanoparticles increased.

Results clearly demonstrate that a newly synthesized Cr_2O_3 nanoparticle was promising antimicrobial agent *Escherichia coli.*

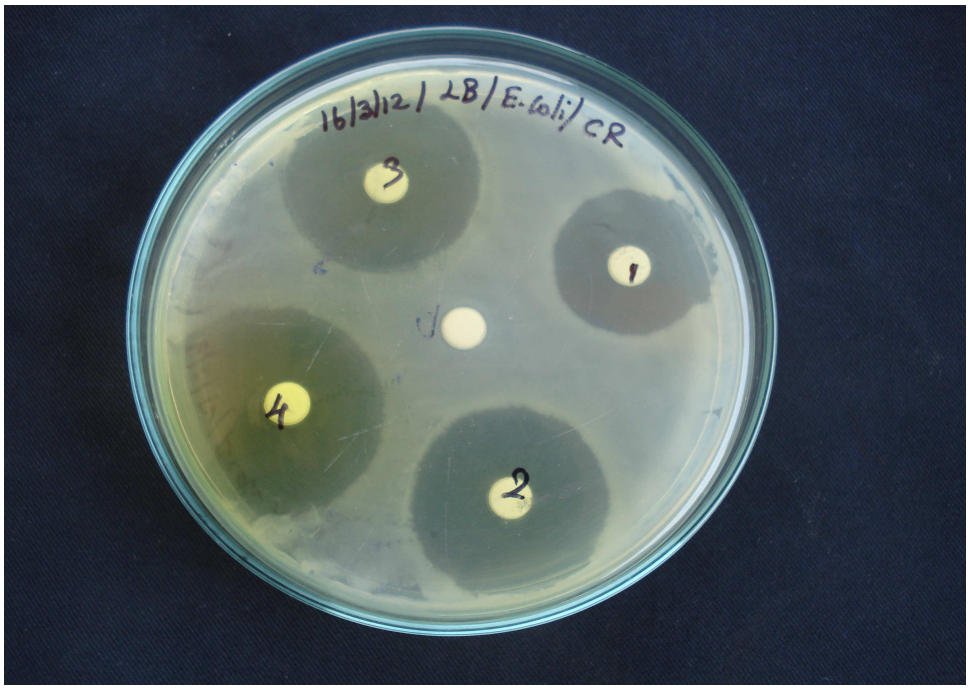

Fig. 5. Representative images of agar plates containing Cr_2O_3 nanoparticle impregnated disks and appearances of inhibitory zones of *Escherichia coli* with different concentrations

CONCLUSION

The waste leaves of *Arachis hypogaea* were utilized in the synthesis of Cr_2O_3 nanoparticles .The reactivity of potassium dichromate solution with *Arachis hypogaea* leaf extract have been studied. The formation of Cr_2O_3 nanoparticles are analyzed using the XRD, SEM, UV-VIS and FTIR studies. The green chemistry approach addressed in the present work on the synthesis of Cr_2O_3 nanoparticles are simple, cost effective, eco-friendly and the resultant nanoparticles are highly stable and reproducible. Significant behavior of Cr_2O_3 nanoparticles reveals that it has effective antibacterial agent against *E. coli*.

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