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Research Article

EXPOSURE CHARACTERIZATION OF Cr₂O₃ NANOPARTICLES SYNTHESIZED BY CANABIS SATIVA LEAVES EXTRACT

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ABSTRACT

Currently, the ecofriendly build out of metal oxide nanoparticles by utilizing plant stuffs has allured substantial recognition. This approach delineates a green technique where Cr₂O₃ nanoparticles were synthesized using chromium nitrate and the deliquescent extract of cannabis sativa as capping and reluctant source. After microscopic characterization it has been seen that the shape of Cr₂O₃ nanoparticles is trigonal. Serial dilution method was adopted to test the antimicrobial activity of Cr₂O₃ nanoparticles, Cr(NO₃)₃, leaves extract and standard drugs against bacteria and fungi. According to the antimicrobial activity results procured the Cr₂O₃ nanoparticles were found to be more potent in the comparison of the standard drugs.

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INTRODUCTION

A propitious way to obtain high yield, environment friendly, low cost and sustainable methods for the synthesis of metal NPs is to exploit the array of biological resources in nature. Indeed, over the years, plants, fungi, viruses, algae, bacteria have been used for production energy efficient, low cost and innocuous metallic NPs. In phytofabricated method NPs synthesis was carried out without addition of any reducing agent and the stabilizer which are replaced by molecules are produced by plant extract, yeast, fungi, bacteria etc. Biosynthesis of metal NPs, using plant leaf extract both have capping and reducing agent, is recently under exploitation. Currently, Cr₂O₃ NPs have gained much scrutiny because of their significant role in science as well as in technology. Leaves extract of Cannabis species contains alkaloid, flavonoids, tannins, phenols, cardiac glucosides, terpenes and steroids, resins volatile oils, and balsam. Hemp is a plant with versatile qualities, capable of producing large biomass quantities in a short duration. Its seeds are consumed as a source of dietary oil while its stem fibrous is used in construction and automotive industries. Also, its leaves and flowers act as a source of bioactive components. Cr₂O₃ NPs boasts peculiar employed applications like corrosion resistant materials [1], high temperature resistant material [2], green pigment [3], coating

materials [4,5], heterogeneous catalysts [6,7] and liquid crystal displays [8]. The structure, composition, size, crystalline and morphology of the inorganic substances fairly reveals their innate properties. The investigation of different chromium NPs synthesis has been shown a strong endeavour [9-11]. The synthesis, characterization and antimicrobial activities of Cr₂O₃ NPs against Klebsiella pneumoniae had been studied and observed that the bacterial growth reduces significantly with the increase in the concentration of Cr₂O₃ NPs [12] and also studied the anti bacterial activities of chromium oxide nanoparticles against enterococcus faecalis and found that Cr₂O₃ nanoparticles shows good antimicrobial activities [13]. The synthesis of chromium oxide nanoparticles via photosynthesis method by using three complexes as a source of chromium was studied and observed that a large quantity of regular nanoparticles are trigonal in shapes and have a size less than 100 nm [14]. Synthesis and characterization of the Cr₂O₃ NPs by Mukia maderaspatana and Mulberry leaves extract was analyzed and found that they can be used for various applications such as catalyst, pigment and antibacterial effect [15].

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Experimental

Materials

All chemicals used in the present study are of high pureness and are procured from Sigma (Bangalore, India). Leaf of plant Cannabis sativa is collected from farm and washed with distilled water, dried, crushed and then extracted with the help of soxhlet extraction apparatus.

Preparation of plant leaves extract

The collected plants leaves were sterilized carefully with distilled water so that necrotic and epiphytes plants can be removed and were dried in dark for ten days. Then, they were grinded into a fine powder with the help of home blender. For the plant broth preparation, the 10 gm of dried powder was boiled with 100 ml of deionized distilled water. The resulting solution was filtered rigorously until no insoluble material appeared in the broth.

Synthesis of Cr₂O₃ NPs

For the preparation of Cr₂O₃ NPs, 20 ml of the plants leaves extracts was added to 80 ml of 1 mM Cr(NO₃)₃ solution in a 250 ml flask and was kept in boiling water bath for 10 min. Now, it was filtered with whatman filter paper No.1 and was kept at different temperature like 40 °C, 50 °C, 60 °C. During temperature treatment, the colour of reaction mixture changes from green to brown which confirms the reduction of Cr₂O₃ nanoparticles given in figure 1.

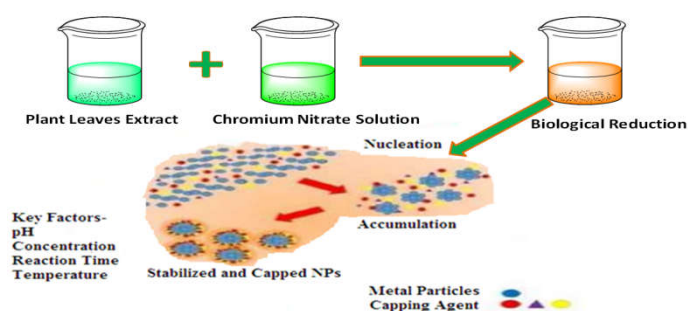


Fig 1 Synthesis of Cr₂O₃ NPs

Characterization Techniques

Fourier Transform Infrared Spectroscopy

Vertex 70 V, Bruker 400-4000 cm⁻¹ was used to explore the FTIR spectra by applying KBr pellet method. For the sample preparation, a small amount of solid KBr was mixed with precipitate of the Cr₂O₃ NPs.

Transmission Electron Microscopy

A transmission electron microscope (FEM TECNAI G2 F30 TEM) operated at 300 kV was used to gather the TEM images of the Cr₂O₃ nanoparticles. The TEM samples were developed by fixing a drop of Cr₂O₃ nanoparticles dispersed in ethanol onto a continuous carbon-coated copper grid. The size and shape of the nanoparticles were examined according to the images.

Scanning Electron Microscope

An optical impression of the surface morphology of the Cr₂O₃ NPs was obtained by SEM. Smart SEM V. 5.05 software was used with Carl Zeiss Merlin Field Emission Scanning Electron

Microscope (FESEM). A high definition backscattered electron (HDBSD) mode was executed in which inorganic substances are lighter in colour than organic compounds. For the elemental composition of the Cr₂O₃ nanoparticles surface the energy-dispersive X-ray spectroscopy (EDS) was used.

Pharmacological Evaluation of Antimicrobial Activity

Antimicrobial activity of Cr(NO₃)₃, Cr₂O₃ NPs and Cannabis sativa leaves extract on each microbes (bacteria such as *B. subtilis*, *S. typhi*, *S. aureus*, *E. coli* and fungi *A. niger*, *A. flavous*, *F. species*, *P. triticena*.) was scrutinized by using DMSO as a solvent and streptomycin, penicillin as standard drugs by standard disc-diffusion method and serial dilution method[16-18]. The incubation of test plates was executed at 37 °C and was examined for zone of inhibition (clear area). The diameter of the percentage of the zone of inhibition was measured using a meter ruler after 24 hrs and denoted in mm. For the calculation of growth of inhibition percentage the underneath expression was considered.

$$\% \text{ inhibition} = (C - T)/C \times 100 \quad (I)$$

Where T = diameter of the microbial colony in test plate.

C = diameter of microbial colony in millimeter in control plate.

RESULT AND DISCUSSION

Fourier Transform Infrared Spectroscopy

Fourier transforms infrared (FT-IR) spectroscopy is highly responsive in indentifying inorganic and organic species with low content. Figure 2 represents the FT-IR spectrum of cannabis stabilized Cr₂O₃ NPs. The FTIR spectrum was recorded in the range of 4000-400 cm⁻¹. and shows characteristic peaks. From the data procured, the peak perceived at 2920 cm⁻¹ can be attributed to the C-H stretching existing in the Cr₂O₃ NPs. The weak broad band obtained at 2935 cm⁻¹ corresponds to Ph-OH group of cannabis molecule. Because of interaction with Cr₂O₃ nanoparticles the C=O stretching of cannabis at 1620 cm⁻¹ get relocated to a greater wave number at 1700 cm⁻¹. The aromatic unsaturation (C=C) of stabilized cannabis molecule is confirmed by three characteristic peaks in occurring between 1570 – 1525 cm⁻¹. The peaks noticed at 1035 cm⁻¹ and 1165 cm⁻¹ signifies to (C-O) band which belongs to cannabis. The characteristic stretching bonds O-Cr-O which indicates the presence of the Cr₂O₃ nanoparticles in the sample is attained at a remarkable absorption peak visible at 725 cm⁻¹.

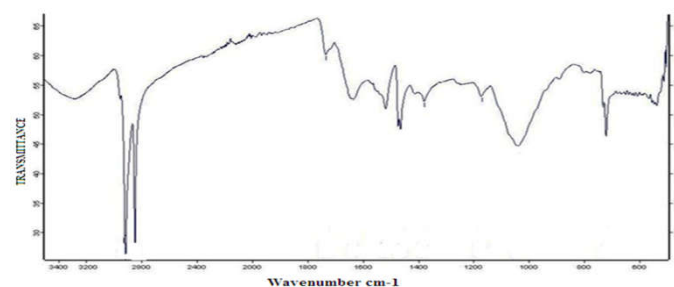


Fig 2 IR spectra of Cr oxide NPs

Scanning Electron Microscope

SEM analysis was performed for the study of morphology of synthesized Cr₂O₃ NPs. The agglomeration of Cr₂O₃ NPs

eventuated during the synthesis process and can be noticed in the SEM images shown in Figure 3. It is clear that the synthesized Cr_2O_3 NPs are fairly dispersed and little agglomerated. From the SEM images of these compounds it is evident that most of the particles have polymorphic morphology. The Cr_2O_3 NPs had acquired trigonal shape and attained around 19 nm of average particle size.

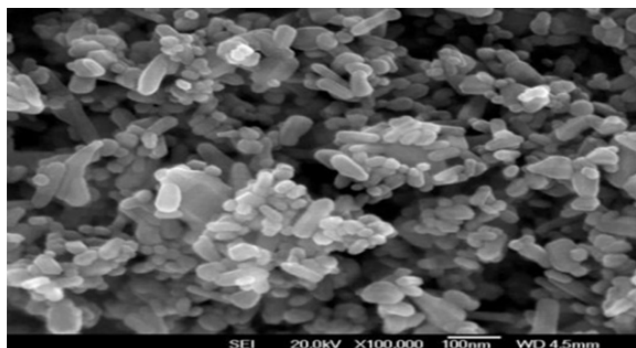


Fig 3 SEM Image of Cr_2O_3 NPs

Transmission Electron Microscopy

TEM image of the Cr_2O_3 nanoparticles is displayed in Figure 4. Electron diffraction patterns were cumulated by HR-TEM to determine the phase and particle size of nanoparticles. It is apparent from the images that the particles developed are of approximately trigonal and eclipse morphology. It is clear from the image of TEM that the shape of Cr_2O_3 NPs was trigonal and size was nearly around 19 nm.

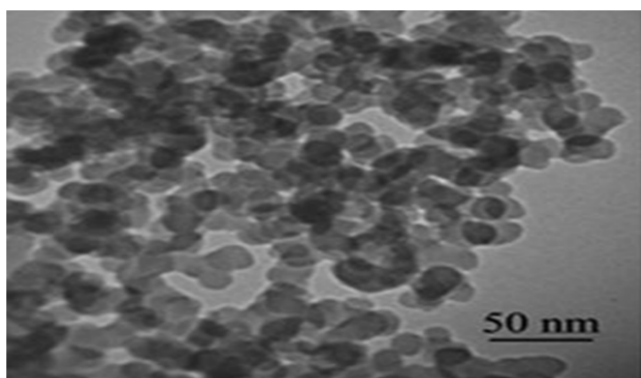


Fig 4 TEM Image of Cr_2O_3 NPs

Energy-dispersive X-ray spectroscopy

A standard EDX spectrum of nanoparticles is displayed in figure 5. Two peaks are clearly visible between 1 kev and 9 kev which represents the chromium characteristics line. Maxima noticed at 3 kev on the left part of the spectrum indicates the carbon peak. A slightly noticeable characteristics line located at 0.5 kev indicate the presence of oxygen. The existence of alkyl chain in stabilizers is assured by the carbon and oxygen peaks in the examined nanoparticles. During EDX studies the spectra obtained were used to attain quantitative analysis and found high chromium contents (80 %) in the analyzed samples. Absorption of strong chromium signal along with other elements has been noticed during EDX characterization which might have originated from the biomolecules that are linked to the surface of Cr_2O_3 NPs.

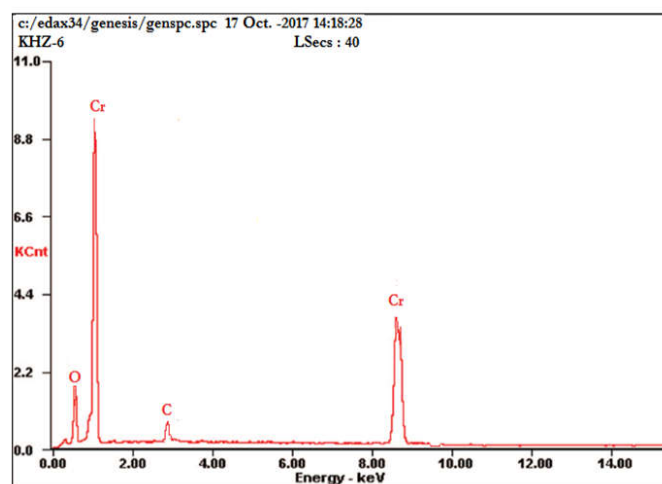


Fig 5 EDX data of Cr_2O_3 NPs

Antimicrobial Activity

The antimicrobial activity of $\text{Cr}(\text{NO}_3)_3$, Cr_2O_3 NPs and Cannabis sativa leaves extract and standard drug was screened in vitro against selected fungi and bacteria. Figure 6 displays the microbial action of the Cr_2O_3 NPs against *S. aureus* (bacteria) and *P. triticina* (fungus). Figures (7, 8) and tables (1, 2) displays the microbial activities of the Cr_2O_3 NPs, Cr_2O_3 , Cannabis sativa leaves extract and standard drug against bacteria and fungi. In accordance with an antimicrobial outcomes active zone of inhibition of $\text{Cr}(\text{NO}_3)_3$, Cr_2O_3 NPs, Cannabis sativa leaves extract and a standard drug was 500 ppm. Also, it asserted that the Cr_2O_3 NPs have pronounced antimicrobial effect against *S. aureus* (bacteria) and *P. triticina* (fungus) in comparison to other compounds. The fabricated Cr_2O_3 NPs were more effective as compared to $\text{Cr}(\text{NO}_3)_3$ solution, Cannabis sativa leaves extract and standard drugs.



Fig 6 Microbial action of Cr_2O_3 NPs

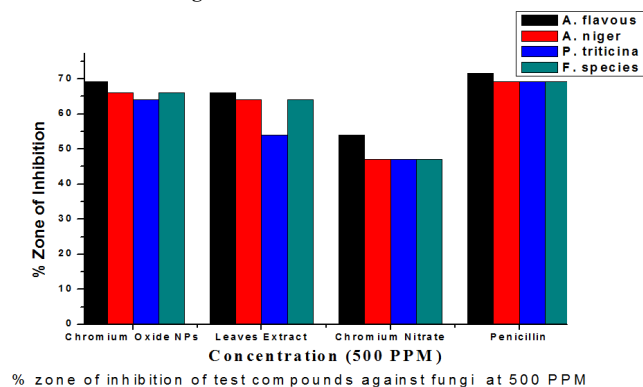


Fig 7 % zone of inhibition of test compounds against fungi at 500 ppm

Table 1 Percentage of zone of inhibition of test compounds against bacteria

Compounds	E. coli				S. typhi				S. aureus				B. subtilis			
	% Conc. in ppm				% Conc. in ppm				% Conc. in ppm				% Conc. in ppm			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Cr ₂ O ₃ NPs	47.13	64	69.13	71.55	47.13	69.13	71.55	75	39.5	66	69.13	75	47.13	66	69.13	71.55
Leaves Extract	39.5	54	64	69.13	39.5	66	69.13	71.55	39.5	64	64	71.5	39.5	64	66	69.13
Cr(NO ₃) ₃	39.5	47.13	71.55	75	39.5	54	64	66	39.5	47.13	54	66	39.5	47.13	64	66
Streptomycin	47.13	69.13	64	69.5	47.13	71.55	75	75	47.13	69.13	71.55	75	47.13	69.13	71.55	75

Table 2 Percentage of zone of inhibition of test compounds against fungi

Compounds	A. flavous				A. niger				P. triticina				F. species			
	% Conc. in ppm				% Conc. in ppm				% Conc. in ppm				% Conc. in ppm			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Cr ₂ O ₃ NPs	47.13	69.13	71.55	75	47.13	69.13	71.55	75	47.13	66	69.13	71.55	39.5	66	69.13	75
Leaves Extract	39.5	66	69.13	71.55	39.5	66	69.13	71.55	39.5	64	66	69.13	39.5	64	66	69.13
Cr(NO ₃) ₃	39.5	54	64	66	39.5	64	66	69.13	39.5	47.13	54	66	39.5	47.13	54	66
Penicillin	47.13	71.55	75	75	47.13	69.13	71.55	75	47.13	69.13	71.55	75	47.13	69.13	71.55	75

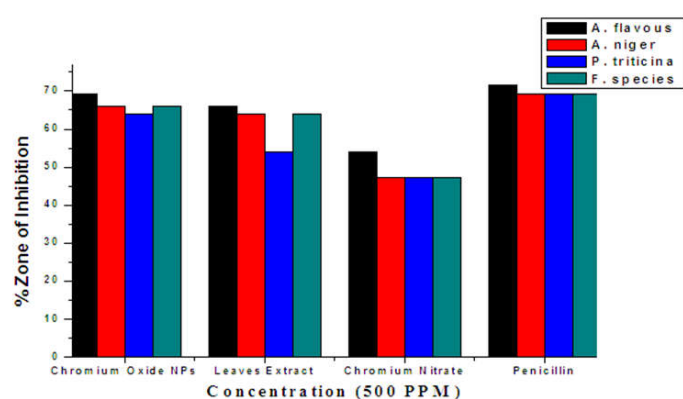


Fig 8 % zone of inhibition of test compounds against bacteria at 500 ppm

CONCLUSION

The green synthesis of Cr₂O₃ NPs is an appealing subject for ongoing scientific temptation and have been enormously explored in materials sciences, because of their physical-chemical properties and their varied range of applications as high temperature ceramics, semiconductors, magnetic materials, coating materials, a humidity sensor, catalysts, super hard materials etc. We can say, it is time for a vast search for the preparation techniques of Cr₂O₃ NPs by plants leaf extract and its use as a corrosion inhibitor. This method can have the benefits of being less expensive with no undesirable by products, simplicity, and it is eco-friendly. Possibly this procedure is operable on a large scale production. The developed Cr₂O₃ nanoparticles had attained particle size of 19 nm and were trigonal in shape. The result of EDX studies reveals that Cr₂O₃ NPs powder contains micrometrical conglomerates which have substantial amount of chromium (about 80%). The ultimate conclusion gathered from the antimicrobial studies assert that as the concentration of the Cr₂O₃ NPs elevates above 500 ppm, the inference almost remains unaltered or is somewhat increased. The zone of inhibition and graphical data inferred that the Cr₂O₃ nanoparticles are microcidal at high concentration and micro static at low concentration. So these nanoparticles are supposed to act as inhibitory for microbial contamination.

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