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Facile Synthesis of Cr_2O_3 Nanoparticles with High Antimicrobial Activity

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ABSTRACT

Currently, the development of green metal NPs through ecofriendly technique has become a major focus of researchers. The current study highlights the antimicrobial activity of Cr_2O_3 NPs, synthesized by *Cannabis sativa* (Bhang), *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) plants leaves extract. The characterization of Cr_2O_3 NPs was attained by SEM with EDX, TEM and XRD analysis. Moreover, synthesized Cr_2O_3 NPs were evaluated for their antimicrobial activities against fungi such as *P. triticena*, *A. niger*, *A. flavous*, *F. species* and bacteria such as *S. aureus*, *B. subitilis*, *E. coli* and *P. fluorescence*. The Cr_2O_3 NPs synthesized by *Cannabis sativa* leaves extract were found to exhibit high antimicrobial activities towards all microbial species. The Cr_2O_3 NPs shows a clear zone of inhibition at concentration 500 ppm. The results revealed that *P. triticena* was tremendously sensitive while interacting with Cr_2O_3 nanoparticles. The outcomes of antimicrobial studies divulged that the Cr_2O_3 NPs are more potent in comparison to the metal salts as well as plants leaves extract. Therefore, the Cr_2O_3 NPs are supposed to be great antimicrobial agents.

1. Introduction

The term 'green synthesis' emanated from the elevating environmental concern which had instigated the researches to ploy the technique of synthesizing the nanoparticles from biological system such as fungi, bacteria and plants. In recent years, the metal nanoparticles synthesized from the plant leaves extract have found to be a promising antimicrobial material. Plants extract contains phytochemicals such as tannin, flavonoids, alkaloids, terpenoids, polyphenols, glycosides, anthraquinones, amino acids, proteins and other heterocyclic compounds which have great antioxidant and antimicrobial properties [1].

Ocimum sanctum commonly known as basil or tulsi is a grassy aromatic perennial plant. It is native to the Indian subcontinent, Afghanistan and Iran [2-4]. It is a traditional medicinal plant able to cure headache, diarrhea, warts, cough, constipation, kidney malfunctions, ulcers and nasal polyps [5]. Moreover, it has been reported to act as nematicide, insecticide, fungicide and antimicrobial compound [6-10]. *Azadirachta indica* commonly known as neem is an evergreen and fast-growing tree. It is native to the Indian subcontinent and found in tropical and semi tropical regions. It is a versatile medicinal plant reported to exhibit antibacterial, antiviral, anti-carcinogenic, anti-inflammatory, immunomodulatory, anti-hyperglycaemic, antiulcer, antimalarial properties [11-19]. *Cannabis sativa* commonly known as bhang is a unique versatile plant. Capable to behave as anti-inflammatory, anticonvulsive, sedative anti-emetic, analgesic have laxative actions [20].

The Cr_2O_3 NPs have great applications such as green pigment [21], corrosion resistant [22], liquid crystal displays [23], high temperature resistant material [24], coating materials [25, 26] and heterogeneous catalyst [27, 28]. Sangwan et al, have synthesized Cr_2O_3 NPs by reduction of potassium dichromate solution with *Arachishy pogaea* leaf tested bactericidal effect against *E. coli* and *Enterococcus faecalis* where they had been analyzed and found that these particles are effective bactericide [29]. The synthesis and characterization of the chromium oxide nanoparticles by *Mukia maderaspatana* and Mulberry leaves extract was studied and observed that produced nanoparticles can be used for various applications such as pigment, catalyst and antibacterial effect [30]. The synthesis of chromium oxide by using three complexes as a source of chromium via photosynthesis method was analyzed and found that the particles obtained were spherical in shape and have a size less than 100 nm [31]. Also, the synthesis, characterization and investigation of antibacterial activity of Cr_2O_3 NPs against klebsiella pneumonia was studied and revealed that these nanoparticles have effective antibacterial activity [32]. The current study was aimed to synthesize Cr_2O_3 NPs using plant leaves extract of *Cannabis sativa* (Bhang), *Azadirachta indica* (Neem), and *Ocimum sanctum* (Tulsi) and to draw attention towards their antimicrobial activity.

2. Experimental Methods

2.1 Plant Specimen

The fresh plants leaves were washed thoroughly with the help of distilled water to remove the adhering soil and dust. Thereafter, the leaves were dried in dark over a period of 10-15 days and were grinded into fine powder with the help of grinder. The powdered specimen was sealed in a plastic bag and kept at room temperature for further use.

2.2 Preparation of Extract from Plants Leaves

A sufficient amount of plant material (powder) was filled in the porous cellulose thimble. (Here, an average of 20 g extract in a 25x80 mm thimble was used). Thimble is placed inside the Soxhlet extractor. Thereafter, solvent, 500 mL of water was added to a round bottom flask, which is connected to a Soxhlet extractor and condenser on an isomantle. The side arm was lagged with glass wool and with the help of isomantle the solvent was heated to evaporate, so that it moves through the apparatus to the condenser. Here, the condensate drips into the container containing the thimble. Once the amount of solvent was upto the siphon it drained into the flask and also the method begins once more. Once the method was completed, the water was dried by a rotary evaporator, leaving a little extracted material (about 2-3 mL) within the flask.

2.3 Preparation of Cr_2O_3 NPs

After mixing plant leaves extract powder (5 g) with ethanol (100 mL), heated for 2 hours at 60 °C. The filtrate was then collected after the filtration process using Whatman filter paper (No.1). 10 mL of ethanolic leaves extract was mixed with 90 mL of 1 mM chromium nitrate aqueous solution in 500 mL beaker. Then one-hour heat was given to that reaction mixture at 80 °C. The greenish coloured solution turned into brown which confirmed the formation of chromium oxide nanoparticles shown in Fig. 1.

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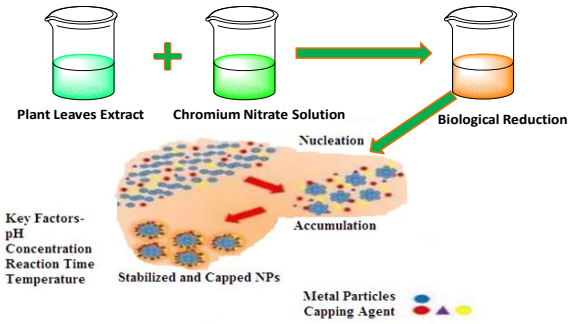

Fig. 1 Synthesis of Cr_2O_3 NPs

2.4 Testing Antimicrobial Effect

To test the antimicrobial activity of compounds the disc diffusion method [33, 34] was used and was screened via Mullar Hinton Agar (MHA) procured from Hi-media (Mumbai). For the preparation of MHA plates, 15 mL of molten media was poured into sterilized petriplates and were allowed to coagulate for 5 minutes. Thereafter, 0.1% inoculumns suspension was swabbed uniformly and was left to dry for 5 minutes. The 40 mg/disc concentrated extract was loaded on 6 mm sterile disc and was positioned on the surface of medium. Here, the extract was left to spread for 5 minutes. After that, the plates were kept for incubation at 37 °C for 24 hr. The measurement of inhibition zones formed around the disc was done with the help transparent ruler in millimeter at the end of incubation.

2.5 Characterization Techniques

Structural and morphological characterizations of the Cr_2O_3 nanoparticles were determined by using Fourier Transform Infra-Red spectroscopy (FTIR) (Thermo-USA, FTIR-380) in the wavelength range of 400 - 4000 cm^{-1}, FESEM Smart SEM V. 5.05 software along with Carl Zeiss Merlim.

3. Results and Discussion

FTIR spectroscopy is a very crucial technique for evincing the organic and inorganic species with low contents. The Figs. 2-4 delineate the FTIR spectra of Cr_2O_3 nanoparticles synthesized by *Cannabis sativa, Ocimum sanctum* and *Azadirachta indica* plants leaves extract. FTIR spectra of all as synthesized Cr_2O_3 nanoparticles have the sharp, intense characteristic absorption band in the range of 400 – 600 cm^{-1} which represents the Cr-O bond. The other weak absorption bands in between the range of 3280 – 3440 cm^{-1} corresponds to hydroxyl group of water.

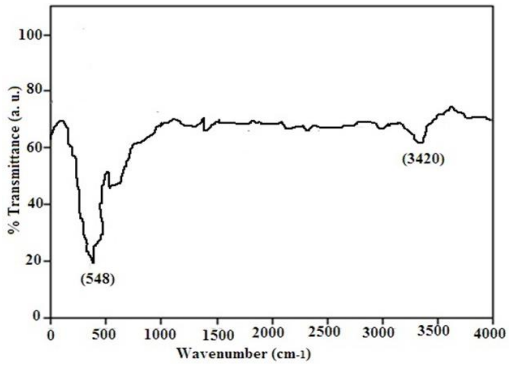

Fig. 2 FTIR spectrum of Cr_2O_3 NPs synthesized by *Cannabis sativa* leaves extra

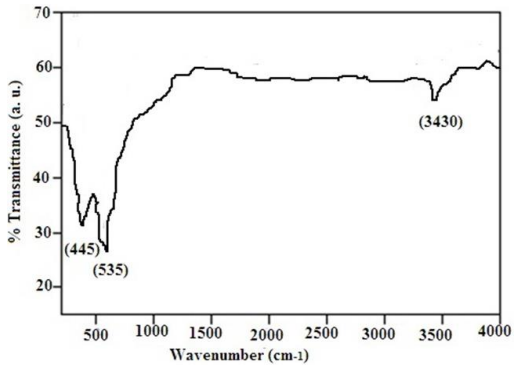

Fig. 3 FTIR spectrum of Cr_2O_3 NPs synthesized by *Ocimum sanctum* leaves extract

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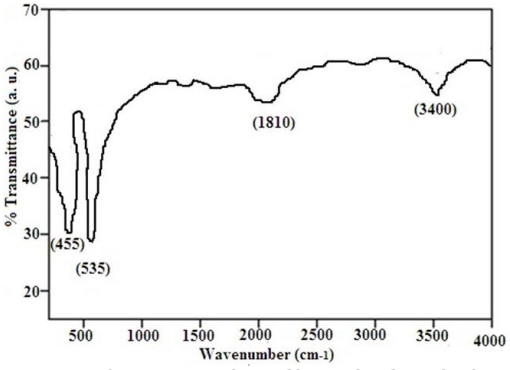

Fig. 4 FTIR spectrum of Cr_2O_3 NPs synthesized by *Azadirachta indica* leaves extract

The morphology of as synthesized Cr_2O_3 nanoparticles using the plant extract *Cannabis sativa* was ascertaining using SEM. It is clear from Fig. 5 that during the synthesis process the agglomeration of the Cr_2O_3 nanoparticles occurred. The synthesized Cr_2O_3 nanoparticles can be seen fairly dispersed and slightly agglomerated in the SEM view. The nature of most of the particles manifests polymorphic morphology. An average particle size of around 34 nm was procured by the Cr_2O_3 nanoparticles and had acquired the oval shape.

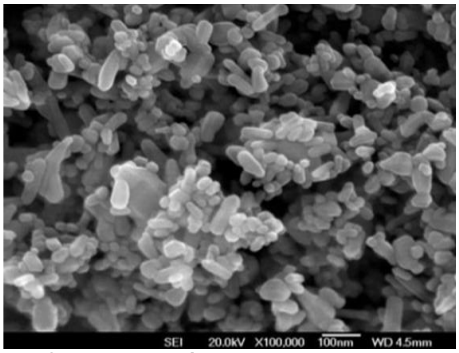

Fig. 5 SEM image of Cr_2O_3 nanoparticles

Fig. 6 delineates about the standard EDX spectrum of as synthesized Cr_2O_3 nanoparticles synthesized by *Cannabis sativa* leaves extract. Four peaks are clearly visible in the range of 0 to 10 kev. The peaks at around 1.2 kev and 8.7 kev represents the chromium characteristics line. The carbon and oxygen peaks are noticed at around 3 kev and 5 kev respectively on the left part of the spectrum. Quantitative analysis was performed using EDX spectra and during analysis 80% of chromium content was attained in the samples. Other synthesized nanoparticles also show the similar EDX spectra.

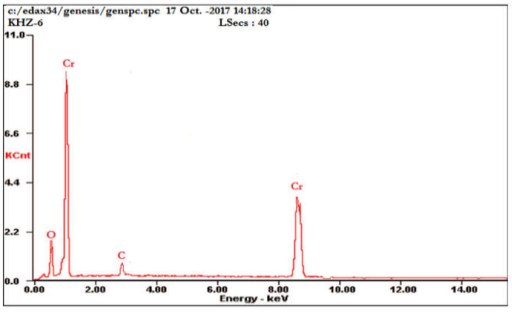

Fig. 6 EDX data of Cr_2O_3 nanoparticles

On the basis of the result of the disc diffusion test, all the four bacteria had shown varying different susceptibilities towards the test compounds. The diameters of the zone of inhibition for all bacteria were determined to range between 9.0 to 21 mm. When the higher concentration of test compounds impregnated disc was used, the inhibition zones were increased for all the bacteria. While at 500 ppm, the inhibition zone was considerably noticed from the lower concentration. At the 500-ppm concentration, the Cr_2O_3 NPs produced the largest zone of inhibition 16 mm for S. *aureus* and 14 mm for rest of three *i.e., E. coli, S. typhi* and *B. subtilis* after 24, 48 and 72 hours (Tables 1-3). Subsequently, the zone of inhibition of test compounds was evaluated against all the fungi. At 500 ppm concentration, the Cr_2O_3 NPs produced the largest zone of inhibition 18 mm for P. *triticina* and was 15 mm, 16 mm and 14 mm for *A. flabous, A. niger* and *F. species* respectively after 24, 48 and 72 hours (Tables 4-6).

Table 1 Zone of inhibition (mm) of test compounds against bacteria after 24 hr (conc. in ppm)

Compounds	*E. coli*				*S. typhi*				*S. aureus*				*B. subtilis*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	4	6	7	8	4	6	7	8	4	6	7	8	4	6	7	8
Leaves Extract b	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7
Leaves Extract c	4	6	7	8	4	6	7	8	4	6	7	9	4	6	7	8
Cr_2O_3NPs a	9	14	15	16	10	14	15	16	9	16	17	18	9	14	15	16
Cr_2O_3 NPs b	9	13	14	15	9	13	14	15	9	14	15	17	9	13	14	15
Cr_2O_3NPs c	9	13	14	15	9	13	13	14	9	13	14	15	9	12	13	14
$Cr(NO_3)_3$	3	5	5	6	3	5	5	6	3	5	5	6	3	5	5	6
Streptomycin	20	25	28	30	20	25	28	30	20	25	28	30	20	25	28	30

Note: *a= Cannabis sativa (Bhang), b= Azadirachta indica (Neem), c= Ocimum sanctum (Tulsi)*

Table 2 Zone of inhibition (mm) of test compounds against bacteria after 48 hr (conc. in ppm)

Compounds	*E. coli*				*S. typhi*				*S. aureus*				*B. subtilis*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	5	7	8	9	5	7	8	9	5	7	8	9	5	6	7	8
Leaves Extract b	5	6	7	8	5	6	7	8	5	7	8	9	5	5	6	7
Leaves Extract c	5	6	7	8	5	7	8	9	5	7	8	9	5	6	7	8
Cr_2O_3NPs a	10	16	16	17	11	16	17	17	11	17	18	19	11	16	17	17
Cr_2O_3 NPs b	10	15	15	16	10	15	16	17	10	16	17	17	10	15	16	16
Cr_2O_3NPs c	10	14	15	16	10	15	15	16	10	15	16	17	10	14	15	15
$Cr(NO_3)_3$	4	6	7	8	4	6	7	8	4	6	7	8	4	5	6	7
Streptomycin	22	28	30	32	22	28	30	32	22	29	31	33	22	27	29	31

Table 3 Zone of inhibition (mm) of test compounds against bacteria after 72 hr (conc. in ppm)

Compounds	*E. coli*				*S. typhi*				*S. aureus*				*B. subtilis*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Leaves Extract b	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Leaves Extract c	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Cr_2O_3NPs a	11	17	18	19	12	17	18	18	12	18	19	20	11	17	18	18
Cr_2O_3 NPs b	11	16	17	18	11	15	17	17	11	17	18	18	11	16	16	17
Cr_2O_3NPs c	11	15	16	17	11	16	16	17	11	16	17	18	11	15	15	16
$Cr(NO_3)_3$	5	6	7	8	5	7	8	9	5	7	8	9	5	6	7	7
Streptomycin	22	28	30	32	22	28	30	32	22	29	31	33	22	27	29	31

Table 4 Zone of inhibition (mm) of test compounds against fungi after 24 hr (conc. in ppm)

Compounds	*A. flabous*				*A. niger*				*P. triticina*				*F. species*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	4	6	7	8	4	6	7	8	4	6	7	8	4	6	7	8
Leaves Extract b	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7
Leaves Extract c	4	6	7	8	4	6	7	8	4	6	7	9	4	6	7	8
Cr_2O_3NPs a	9	15	16	17	9	16	17	18	10	18	19	20	9	14	15	16
Cr_2O_3 NPs b	9	14	15	16	9	15	16	17	9	16	17	17	9	13	14	15
Cr_2O_3NPs c	9	14	15	15	9	14	15	16	9	16	16	17	9	13	14	15
$Cr(NO_3)_3$	3	5	5	6	3	5	5	6	3	5	5	6	3	5	5	6
Streptomycin	20	25	28	30	20	25	28	30	20	25	28	30	20	25	28	30

Table 5 Zone of inhibition (mm) of test compounds against fungi after 48 hr (conc. in ppm)

Compounds	*A. flabous*				*A. niger*				*P. triticina*				*F. species*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	5	7	8	9	5	7	8	9	5	7	8	9	5	6	7	8
Leaves Extract b	5	6	7	8	5	6	7	8	5	7	8	9	5	5	6	7
Leaves Extract c	5	6	7	8	5	7	8	9	5	7	8	9	5	6	7	8
Cr_2O_3NPs a	10	16	17	18	10	17	18	19	11	19	20	21	10	15	16	17
Cr_2O_3 NPs b	10	15	16	17	10	17	17	18	10	17	18	18	10	14	15	16
Cr_2O_3NPs c	10	15	16	16	10	16	16	17	10	17	17	18	10	14	15	15
$Cr(NO_3)_3$	4	6	7	8	4	6	7	8	4	6	7	8	4	5	6	7
Streptomycin	22	28	30	32	22	28	30	32	22	29	31	33	22	27	29	31

Table 6 Zone of inhibition (mm) of test compounds against fungi after 72 hr (conc. in ppm)

Compounds	*A. flabous*				*A. niger*				*P. triticina*				*F. species*			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Leaves Extract a	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Leaves Extract b	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Leaves Extract c	6	8	9	10	6	8	9	10	6	8	9	10	6	8	9	10
Cr_2O_3NPs a	11	17	18	19	11	19	20	21	12	21	22	23	11	16	17	18
Cr_2O_3 NPs b	11	16	17	18	11	18	18	19	11	18	19	19	11	15	16	17
Cr_2O_3NPs c	11	15	16	17	11	17	18	18	11	18	18	19	11	15	16	16
$Cr(NO_3)_3$	5	6	7	8	5	7	8	9	5	7	8	9	5	6	7	7
Streptomycin	22	27	30	32	22	28	30	32	22	29	31	33	22	27	29	31

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Here, Cr_2O_3 NPs synthesized from *Cannabis sativa* had found to exhibit leading zone of inhibition for microbes than Cr_2O_3 NPs synthesized from others and was more sensitive towards *S. aureus* and *P. triticina*.

Recently, the products obtained from the biological systems such as fungi, bacteria and plants are in substantial exigency because of their immense and considerable biological belongings which includes the therapeutic and commercial efficacy. The conventional medicinal plants such as *Cannavis sativa, Azadirachta indica* and *Ocimum sanctum* contain antimicrobial properties and show inhibitory activity against certain microbes. These plants are rich in phytochemicals such as terpenoids, polyphenols, flavonoides, alkaloids, tannin, glycosides, anthraquinones, amino acids, proteins and other hetrocyclic compounds.

These plants contain major phenolic groups which have antimicrobial activity, anticarcinogenic, and anti- inflammatory properties and are reported by various researchers [35-37]. Because of these properties the metal nanoparticles synthesized from these plant leaves extract have tendency to adsorb on the bacterial cell and undergo dehydrogenation at the cell membrane of bacteria due to respiration process. The enzymes of bacteria get inactivated and hydrogen peroxide is produced causing bacterial cell death [38]. Also, among these the terpenoids are reported to exhibit antifungal activity towards several microorganisms [39,40].

MIC value of Cr_2O_3 NPs synthesized by *Cannabis sativa* is the lowest concentration at which it inhibits the growth. In the other hand MFC value is the lowest concentration at which it can able to kill 99 to 50% of the microorganisms. Here the value of MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) for Cr_2O_3 NPs synthesized by cannabis sativa was procured same (14 mg/mL).

At 500 ppm concentration, the Cr_2O_3 NPs synthesized from C*annabis sativa* had shown supreme zone of inhibition for all microbes than Cr_2O_3 NPs synthesized from *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi) and was more sensitive towards *S. aureus* and *P. triticina*.

4. Conclusion

The purpose of current research work was to synthesize the different Cr_2O_3 NPs by plants leaves extract using green method and to highlight the potential of these nanoparticles as antimicrobial agents which can further be explored for their various applications. It was observed from the result that Cr_2O_3 NPs are more effective against all the microbes in comparison to the metal salts as well as plants leaves extract. Comparisons can be summarized in the following order. Cr_2O_3 NPs a > Cr_2O_3 NPs b> Cr_2O_3 NPs c > leaves extract a > leaves extract b > leaves extract c > $Cr(NO_3)_3$ where a= *Cannabis sativa* (Bhang), b= *Azadirachta indica* (Neem) and c= *Ocimum sanctum* (Tulsi).

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