GREEN SYNTHESIS OF CHROMIUM OXIDE NANOPARTICLES USING CHROMIUM (III) COMPLEX AS A SINGLE ROUTE PRECURSOR: ANTI-OXIDANT ACTIVITY

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ABSTRACT
Green synthesis of metallic nanoparticles using plant sources has become an excellent substitute for conventional chemical synthetic methods. Nowadays, nano biotechnology is growing at a very fast rate due to its various possible application in the pharmaceutical biomedical, textile, paper industries. In our study, we have reported the green synthesis of chalcogenide nanostructure pharmacologically active chromium oxide (Cr$_2$O$_3$) nanoparticles using synthesized chromium (III) complex as a single route precursor. The synthesized chromium (III) complex was reacted with an aqueous extract of cinnamon bark for the green synthesis of Cr$_2$O$_3$ nanoparticles. In the present study, we aimed to synthesize chromium oxide nanoparticles (Cr$_2$O$_3$ NPs) through a facile low-cost, eco-friendly route. In this method, we use the aqueous environment for green synthesis of Cr$_2$O$_3$ NPs because the use of an aqueous medium plays a very important role in reducing time, reducing minimum possibilities of side reactions and proper execution of conversions of synthesized Cr(III) complex into a good quality of Cr$_2$O$_3$ NPs in a very less time. The synthesized chromium (III) complex and green synthesized Cr$_2$O$_3$ NPs were thoroughly analyzed through various structural, morphological, electronic, vibrational and pharmacological characterization techniques. Powdered X-ray diffraction studies confirm the formation of well-defined equip spaced crystalline nanoparticles of chromium oxide. Transmission electron microscopy exhibits oval-shaped structure of Cr$_2$O$_3$ NPs with an average particle size of 48 nm. Sharp electronic absorptions ends at 345nm for Cr(III) complex and at 429nm indicates the synthesis of good quality of chromium(III) complex and Cr$_2$O$_3$ NPs. The FT-IR spectral studies confirmed the presence of Cr-O stretching, N-H bonding and C=O stretching vibrations in synthesized Cr(III) complex was performed to investigate the thermal stability of the complex. The Cr(III) complex is stable up to 350°C. The effective pharmacological activities like in vitro antimicrobial and antioxidant activities explained the presence of strong electron-withdrawing and electron-withdrawing groups in synthesized chromium (III) complex. The green synthesis of Cr$_2$O$_3$ NPs via aqueous extract of cinnamon back in proper stoichiometric ratio is a good method for synthesizing highly effective bioactive agents which will be considered as a good drug candidate for various biological applications in future for various biomedical applications.

Keywords: Cr(III) Complex, Cr$_2$O$_3$ NPs, Green Synthesis, Antibacterial, Antioxidant

INTRODUCTION
Nano biotechnology is a branch in which tools from nanotechnology are developed and applied to study biological phenomenon because nanoparticles serve as probes, sensors vehicles for biomolecule delivery in cellular systems. Nano biotechnology further deals with the green synthesis of cost-effective; eco-friendly, biocompatible and biogenic nanomaterials. The major applications of chromium oxide nanoparticles are in heterogeneous catalyst, liquid crystal displays, wear resistant and high-temperature materials and solar-absorents as well as functional pigments with high reflectivity in the near IR region which is due to their optical properties. In addition, the catalytic applications of chromium nanoparticles have been numerous reactions like toluene oxidation, methanol decomposition and ethane degradation. The Cr$_2$O$_3$ nanoparticles is of highly significant various metallic oxide-based nanoparticles due to their unique physicochemical properties like high melting point, higher stability, wide band gap (-3.4ev), major application in green pigment production. The Cr$_2$O$_3$ NPs have been widely utilized in different industrial applications like catalysis, photonics, coating materials, advanced colorants. The trivalent Cr$_2$O$_3$ nanoparticles are considered the most stable nanoparticles as compared to other chromium oxides. In spite of being a promising compound, few studies have evaluated chromium oxide nanoparticles for various biological applications.
applications because of their potential cytotoxic effects that have been reported in many studies. The major cytotoxic effect of Cr₂O₃ NPs is alternation in the brain and kidneys of rats, as a result of this reactive oxygen species (ROS) production seems to cause a considerable increase in aldehyde (MDA) concentration as well as a significant decline in superoxide dismutase and glutathione levels. The pathological evaluations show deleterious oxidative stress as a consequence of ROS generation. The toxic effects of nanoparticles can be reduced by coating as functionalization of their surfaces with biogenic materials. One of the most promising ways to reduce the cytotoxicity of nanoparticles is surface coating of Cr₂O₃ NPs with plant’s biogenic phytomolecules.

The synthesis of metallic 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 plants is a facile, robust, cost-effective, eco-friendly and easily scalable technique. The green synthesis of nanoparticles via plant sources is an ecofriendly and biocompatible method because, after several rounds of washings, the toxic chemicals cannot easily be removed from the surface of nanoparticles. Therefore, harmful chemicals are present on the surface of nanoparticles, making them less biocompatible and limiting their biological applications. Green synthesis of nanoparticles via plant materials uses phytochemicals as the reducing and capping agent, and no additional chemicals are required. The plant biogenic environment phyto molecules have molecular functionalities that are biologically active and have antibacterial, antifungal, antioxidant, anticancer properties etc. So green synthesis via plant sources enhances the nanoparticles biocompatibility and is responsible for the synergetic effect.

In this work, we synthesized Cr₂O₃ NPs using an aqueous extract of cinnamon bark of a medicinal plant. Cinnamon bark is used for gastrointestinal (GI) upset, diarrhea and gas. It is also used for stimulating appetite, for infections caused by bacteria and parasitic worms; and for menstrual cramps, the common cold, and the flu (influenza) Cinnamon bark, as a part of a multi-ingredient preparation, is applied to the penis for premature ejaculation. In foods, cinnamon is used as a spice and as a flavoring agent in beverages. In industrial applications, cinnamon oil is used in small amounts in toothpaste, mouthwashes, gargles, lotions ointments, soaps, detergents and other pharmaceutical products and cosmetics. Cinnamon bark also contains a chemical that might work like insulin to lower blood sugar. However, these effects are thought to be fairly weak. There are also ingredients in cinnamon bark called tannins that might help wounds by acting as an astringent and also prevent diarrhea. Many reports are available that highlight the biological importance of cinnamon. Till now, many plants have been utilized for the synthesis of Cr₂O₃ NPs. Among the plants used, some are either not biologically active or they are biologically active but have toxic effects. Therefore, Cr₂O₃ nanoparticles for biological applications need to be synthesized with cinnamon are biologically active with no toxic effects. In this regard, cinnamon bark is considered a more prominent part of the cinnamon plant that has important biological properties compared to other parts. In this study, we have utilized cinnamon bark for the green synthesis of Cr₂O₃ nanoparticles. The biosynthesized Cr₂O₃ NPs were further evaluated for antibacterial and antioxidants activities. They have shown excellent antibacterial and antioxidant properties. They exhibited extraordinary antibacterial activity by inhibiting the growth of a broad spectrum of both gram-positive and gram-negative bacterial strains. Moreover, the biosynthesized Cr₂O₃ NPs showed excellent biological activities as compared to chemically synthesized and previously reported Cr₂O₃ nanoparticles.

**EXPERIMENTAL**

Analytical grade chemicals and reagents are obtained from Merck and used as received without further purification. Distilled water was used for all the experiments.

**Spectroscopic Characterization of Compounds**

UV- visible spectra of all synthesized compounds were recorded on a Perkin Elmer UV- Visible Lambda 25 Spectrophotometer in the range of 200-900 nm. FT-IR spectra were recorded in KBR pellets on Perkin Elmer FT-IR spectrophotometer in the range of 4000-400 cm⁻¹. Thermal degradation of compounds was performed on a PCT-thermo balance analyzer in the presence of constant air supply at a heating rate of 1000 C/ minute from room temperature to 1000°C. The conductance measurement of the complex was carried out at room temperature in 10⁻³ M DMSO using Digisun Electronic digital conductivity meter. In this experiment, 0.01 mole potassium chloride solution is used for the calibration of the conductivity.
Antioxidant activities of target compounds were screened against DPPH using in vitro antioxidant protocol. The targeted derivatives of the curcumin molecule were evaluated for their collaborative antimicrobial activity against gram-positive and gram-negative bacterial strains. The zone of inhibition was measured by considering the disc diffusion method. In vitro minimum inhibitory concentrations of targeted compounds were measured using the broth micro dilution method.

**Synthesis of Compounds**
The whole synthesis process was divided into three steps:

1. **Synthesis of Schiff Base ligand**
2. **Synthesis of Coordinated Chromium (III) Complexes from Schiff base ligand via refluxing method.**
3. **Green synthesis of Cr\(_2\)O\(_3\) NPs from coordinated Cr(III) complex**

**Synthesis of Schiff Base Ligand**
Schiff base ligand was prepared by the condensation reaction between acid hydrazide (5 m mol) and aromatic aldehyde (2.5 m mol) in 50 ml of absolute ethanol. The process of synthesis of Schiff base ligand can further be divided into three steps

**Synthesis of Malonic Ester (Precursor -1)**
An equi molar amount of 4-methoxy aniline and diethylmalonate were taken in a three-necked round bottom flask in 1: 1 molar ratio. Shake the solution vigorously and heat the mixture for 30 minutes at 80\(^{\circ}\)C. Cool the solution and add 30 ml of absolute alcohol in the mixture with continuous stirring for 30 minutes. As a result of continuous stirring and on heating a colorless suspension appears in the round bottom flask. After cooling, pour this solution into 100-gram crushed ice with continuous mechanical stirring. After vigorous stirring of contents for 2 hours, a white crystalline precipitate is obtained which was filtered, washed with the ethanol, recrystallized and air-dried.

**Synthesis of Malonanilic Acid hydrazide- (Precursor-II)**
For the synthesis of precursor-II, 10 ml of hydrazine hydrate was added slowly in the ethanolic solution of the malonic ester with continuous mechanical stirring for about 1 hour. The contents were left overnight. After 24 hours of aging process white crystalline product separated which was filtrated, recrystallized and air-dried in a vacuum desiccator over P\(_4\)O\(_10\). The yield of the product was calculated by a simple weighing method and found to be 70%. The melting point was calculated via capillary method using digital melting point apparatus was 186\(^{\circ}\)C.

**Synthesis of Schiff Base Ligand**
For the synthesis of a targeted Schiff base ligand, firstly, malonanilic acid hydrazide (0.01 mmo) was dissolved in 50 ml of absolute ethanol at the temperature of 600\(^{\circ}\)C. Then an equi molar amount (0.01 m mol) of cinnamaldehyde obtained from aqueous extract of cinnamon bark was added in hot absolute alcohol (50 ml) in the separate round bottom flask. Finally, both the solutions were mixed with continuous mechanical stirring at 700\(^{\circ}\)C for 1/2 hours then the resulting mixture was refluxed at 80\(^{\circ}\)C. After 6 hours of refluxing process, the product was cooled at room temperature. After 24 hours aging product was filtered, washed with absolute ethanol, recrystallized from absolute ethanol and dried over silica gel.

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Scheme-2 Synthesis of Malonanilic Acid hydrazide- (Precursor-II)

Scheme-3 Synthesis of Schiff Base Ligand

Dark brown crystals; yield:70%, m.pt.200°C; Analysis calculated for (C₁₉H₁₉N₃O₂): C, 76.80; H, 7.03; N,15.16%; Found: C, 78.80; H, 7.03; N,15.20%; IR (K Br, cm⁻¹); v: 1640 cm⁻¹(N=C), 1274 (C-N), 1196 (C-O); H NMR (300 MHz, CDCl₃) δ: 8.62 (2H, s, N=CH), 6.32-7.60(12H, m, Ar -H), MS m/z Calc. for C₁₉H₁₉N₃O₂ -322 g/mol found -321.

Solubility: Ethanol, DMF, DMSO

Synthesis of Chromium (III) Complex from Schiff Base Ligand

For the synthesis of Cr(III) complex of Schiff base ligand, the prepared Schiff base ligand (1mmol) of aromatic aldehyde was dissolved in absolute ethanol (40 ml) and gradually adding chromium chloride hexa hydrate (0.01 m mol) in 50 ml ethanol with continuous mechanical staring at 70°C for 8 hours. After cooling the contents, colored solid precipitate of Cr (III) complexes formed which was filtered off and washed thoroughly with absolute ethanol, recrystallized and air-dried over silica gel.

Dark brown crystals; yield: 59%, M.P.>300°C; Analysis calculated for C₁₉H₁₉N₃O₂ CrCl₃: C, 61.22; H, 6.01; N, 11.87, Cr 10.6% Found: C, 61.24; H, 6.00; N, 11.84; Cr, 10.8%; IR (K Br, cm⁻¹); v: 1620cm⁻¹ (N=C), 1257 (C-N), 1175 (C-O), 450 (Cr-N);

Solubility: Ethanol, DMF, DMSO, Molar Conductance (DMSO 25°C): Λ m : 11.8 S cm² mol⁻¹

Green Synthesis of Cr₂O₃ Nanoparticles

For obtaining Cr₂O₃nanoparticles, 1 g of Cr (III) complex of Schiff base ligand was dissolved in 30 ml of DMSO in a round bottom flask then add 100 ml of aqueous extract of cinnamon bark with continuous stirring at room temperature for 30 minutes and then was kept for aging for another 30 minutes. The final solution was heated in a microwave oven for 5 minutes with 800W power microwave resulted in immediate formation of Cr₂O₃ nanoparticles The resulting solution containing Cr₂O₃ nanoparticles was then cooled, centrifuged, and washed with absolute methanol several times.

Structural Morphological Characterization of Compounds

For electronic characterization of Cr₂O₃ nanoparticles, nanoparticles were collected and characterized by various spectroscopic, structural and morphological characterization techniques like FT-IR, UV- Visible, XRD, Particle size analysis, TEM and SEM. The structure and particle size of Cr₂O₃ nanoparticles were
characterized by a Bruka axis D8 Phase X-ray diffractometer (XRD) using Cu Kα radiation (λ = 1.540 Å). In the 2θ ranging from 20 to 80°. TEM micrographs were obtained by TECHAI G2F20 operated at 300 KV using a drop of suspension of the sample in ethanol on carbon-coated copper grid. Electronic spectra of Cr₂O₃ nanoparticles on quartz in the range of 200-900 nm were obtained using Perkin Elmer Lambda 25 spectrophotometer.

\[
\text{M:L -1:2 \ [Cr(L1)]2Cl}
\]

Image-1: Structure of Cr (III) Complex obtained from Schiff Base ligand

**Evaluation of In-vitro Antibacterial Efficiency of Synthesized Compounds**

Procurement of MTCC cultures of bacteria from PGI Chandigarh which is *E. Coli* (MTCC-1687), *E. faecalis* (MTCC-439) and *S. aureus* (MTCC-737) and indigenous Methicillin-resistant *S. aureus* isolates was used. Stocks of the experimental compound of concentration of 10mg/ml were prepared in DMSO passed through 0.22 mm disposable syringe filter aseptically for sterilization followed by preparation of successive 5 dilutions using sterile distilled water. For antibacterial activity nutrients agar media plates were prepared for working with bacteria. Bacterial inoculums were taken from the broth of revived cultures using a sterile swab and seeded on to the nutrient agar media followed by punching of 5 wells of 6mm diameter. 10 microliter of different dilution of each compound were poured into every 5 different wells of pre-inoculated culture plates separately with different microbial species. The culture plates were incubated at 37± 1°C for 24 hours respectively. Observations were taken in the form of the zone of inhibition (in mm) after incubation of 24 hours of cultured plates.

**Evaluation of In-vitro Antioxidant Activities of Synthesized Compounds**

The radical scavenging activity of different samples was determined by using DPPH assay according to Chang et al., (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as a reference.

**Principle**

1,1Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

\[
(DPPH)^+ + (H-A) \rightarrow DPPH-H + (A)
\]

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence, the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

**Reagent Preparation**

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

**Working Procedure**

Different volumes (2 - 20μl) of synthesized compounds were made up to 40 μl with DMSO and 2.96 ml DPPH (0.1 m M) solution was added. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the compounds was calculated using the following formula,
%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100

Where; RSA is the Radical Scavenging Activity; \text{Abs control} is the absorbance of DPPH radical +ethanol; \text{Abs -sample} is the absorbance of DPPH radical +sample drug.

**Molecular Docking Studies against the Main Protease (M pro 6LU7) of SARS –CoV-2**

The X-ray crystallographic structure of the main protease (M pro PDB ID 6LU7) of SARS –CoV-2 has been downloaded from the protein data bank (PDB) (http://www.pdb.org) database. Preparation of protein for docking simulation was achieved by using Graphical User Interface Program “Auto Dock tools (ADT) 1.5.6” (Molecular Graphics Laboratory tool as MGL tool) developed by Scripps Researchers Institute. Specific chain length (chain A) of Protein (6LU7) has been selected for the preparation of the receptor protein input file for docking study.

Receptor protein preparation for docking study was initiated by remaining water molecules, heteroatom and co-crystallized ligands from PDB crystal of Protein 6LU7, polar hydrogen atoms along with kollman united atom charges were added subsequently to the receptor protein input file and finally the receptor protein input file was saved as Pdb file. The three-dimensional (3D) structure of ligands and Coordinated chromium (III) complex were drawn in Chem sketch (ACD/ Structure Elucidator, version 12.01, Advanced Chemistry Development, Inc. Toronto, Canada,2014(http://www.acdlabs.com ), MM2 program incorporated in Chem Draw Ultra 8.0 were used for geometry optimization of all the compounds and finally, the geometry of all compounds was further optimized with the help of MOPAC6 package using the semi-empirical AM1 Hamiltonian (88) and structure of all compounds were saved as .Pdb file .The input of Pdbqt file of all the compounds in the docking simulation was generated with the help of Auto Dock Tools (ADT) by assigning the required Gasteiger charge and merging non-polar hydrogen. The current research work is based on the antioxidant activity of Schiff base ligand and its coordinated chromium (III) complex and is to be evaluated against covid-19 with the help of molecular docking studies. Furthermore, the molecular docking study is carried out on Schiff base ligand and its chromium (III) complex to identify the antioxidant capabilities through inhibition of the main protease enzyme. As per our objectives, the three-dimensional crystal structures of thymidylate synthase enzyme (PDB ID: 6LU7) were procured from the protein data bank. Molecular docking study was carried out using Auto dock Vina as per the procedure adopted by Agrawal et al. (2020) and the docking parameters were defined as coordinates of the center of the binding site with x = -22.283, y = 12.599, z = 58.966 and in case of DNA gyrase, binding radius = 1.000 Å and the grid dimension used for all the three (3) proteins are 47.25 × 47.25 × 47.25 Å (grid size) with point separated by 1.00 Å (grid-point spacing).

**Docking Study Using Auto Dock Vina**

Auto dock Vina program 1.1.2 developed by Scripps Research institute were employed for all the molecular docking simulations and BIOVIA. Discovery studies 2020(DS), version 20.1.0.0 (Dassault System BIOVIA, Discovery Studio Modeling Environment, Release 2017, San diego: Dassault Systems 2016) and Edu Pymol version 1.7.4.4 was used for the visualization and analysis of docking results and corresponding intermolecular interactions between receptors and the compounds. In order to eliminate any biases arising during docking study blind docking of the compounds and into the protein were carried out by constructing three dimensional (3D) affinity (grid) maps and electrostatic grid boxes of dimension 50 X 50 X 50 Å grid point s and grid center (X,Y, Z) of -26.283 12.599 58.966 with a spacing of 1.00 Å with the help of Auto grid auxiliary program for each of the receptor to cover the entire active site and essential residues within the binding pocket. Lamarckian generic algorithm was used for all docking simulations and all torsions were allowed to rotate. The molecular docking simulations have been done according to a previous validation study. The receptor and ligands were prepared for docking simulation using Auto dock tools 1.5.6. The receptor and ligands were protonated. The receptor as macromolecule has added the Kollman charges while the ligands have added the Gasteiger charges. The grid parameter file is according to the grid box that comprised of 40×40×40 points with 0.575Å space and was centered on the active site of the receptor (x = 30.010, y = -1.913, and z = 24.207). Auto Dock 4.2 (The Scripps Research Institute) was used to do the molecular docking simulation. The docking parameter file is according to Lamarckian Genetic
Algorithm (LGA) with 100 number runs, 150 population size, 2,500,000 energy evaluation, 0.02 rate of gene mutation, and 0.8 rates of the crossover. The conformation results from the docking simulation were clustered using a root mean square deviation (RMSD) tolerance of 1.0 Å. The ligand conformation with the lowest free binding energy ($\Delta G$) was chosen from the most favored cluster. The best ligand conformation was used for the further step of the analysis. The receptor-ligand complexes from docking simulation were visualized using EduPy MOL 1.7 and BIOVIA Discovery Studio Visualizer 2017.

**RESULTS AND DISCUSSION**

This research work reports the green synthesis of $\text{Cr}_2\text{O}_3$ nanoparticles from synthesized Cr (III) complex using an aqueous extract of cinnamon bark which was free from any impurity and does not require continuous stirring of the reaction mixture. The appearance of colored particles indicates the formation of crystalline $\text{Cr}_2\text{O}_3$ nanoparticles.

**Phytochemical Screening of Aqueous Extract of Cinnamon Bark Extract**

To understand the presence of various phytochemical constituents in the aqueous extract of cinnamon bark extract, the extract was subjected to qualitative phytochemical screening. For this, some specific functional group tests were performed to check the availability of biomolecules like carbohydrates, proteins, flavonoids, alkaloids, glycosides, steroids etc.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phyto constituents</th>
<th>Availability in Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Resins</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Cardiac Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>Phytosterolsand</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>Fixed oil and fats</td>
<td>-</td>
</tr>
</tbody>
</table>

**Visual Characterization of Compounds**

Synthesized Cr (III) complex solution has turned brown in color when freshly prepared aqueous extract of cinnamon bark extract was added in the solution with constant stirring which indicated the formation of $\text{Cr}_2\text{O}_3$ nanoparticles. According to the literature, there are three phases of synthesis of metallic nanoparticles via the green approach. The first phase is the activation phase which indicates metal ion reduction and then the nucleation process starts. This phase is considered the growth phase, which involves the aggregation of biosynthesized small metallic nanoparticles. The last phase is the termination phase which facilitates the final shape and geometry of biosynthesized nanoparticles. In the bio-reduction process of chromium oxide nanoparticles, crystalline Cr(III) complex was dissolved in ethanol. Due to the ionic nature of Cr complex, it immediately dissociates into $\text{Cr}^{3+}$ ions and $\text{Cl}^-$ ions, when freshly prepared aqueous extract of cinnamon bark mixed with ethanolic solution of Cr(III) complex. The various functional groups are present in aqueous extract immediately interact with $\text{Cr}^{3+}$ ions and reduce it to its zero-valent state i.e. Cr$^0$, which leads to the formation of $\text{Cr}_2\text{O}_3$ nanoparticles followed by the growth phase, leaving behind the remaining components as a byproduct.

Brown colored $\text{Cr}_2\text{O}_3$ nanoparticles were obtained using an aqueous extract of cinnamon bark via the ultracentrifugation method. Green synthesis of $\text{Cr}_2\text{O}_3$ nanoparticles provides selective and high quality, uniform size, high reaction rate and ecofriendly method. Green Synthesis of $\text{Cr}_2\text{O}_3$ helps to deliver the...
hydration energy into the reaction contents and increases the speed of reaction and efficiency of conversion of reactants into products. Green Synthesis of nanoparticles reduces the crystallization time of nanoparticles and improves the crystalline pattern or behavior of the final end product is a very specific route. In our work, the green synthesis of Cr₂O₃ nanoparticles helps to obtain good quality hexagonal nanoparticles. The short reaction time in green synthesis of nanoparticles results in increasing the nanoparticles purity by decreasing the unwanted side reactions compared to classical thermal methods.

**Optical Properties**

**UV-Vis Spectroscopy Study**

The optical properties of synthesized nanoparticles were measured at room temperature using UV-Vis and FT-IR spectrophotometer. Very sharp absorption occurs in the range of 200-700 nm. The formation of sharp absorption spectra of nanoparticles at 260-780 nm indicates the formation of good quality nanoparticles formed in the process of bio fabrication of Cr₂O₃ nanoparticles using an aqueous extract of cinnamon bark. A broad absorption peak appeared at 590 nm due to the surface plasmon resonance phenomenon that occurred when Cr₂O₃ nanoparticles were prepared from synthesized Cr(III) complex via Schiff base ligand.

The electronic spectrum of the Schiff base ligand was shifted from 325 nm to 340 nm after coordination with Cr³⁺ ion in a trivalent oxidation state. Due to this shifting in electronic spectra of Schiff base ligand bathochromic shifts was aroused in coordinated Cr(III) complex due to back bonding of d electrons from Cr³⁺ ion to C=N group of Schiff base ligand. This back bonding of d electrons decreased the bond energy of the azomethine group of Schiff base ligand.

**FTIR Spectroscopy Study**

FTIR spectroscopic study of synthesized compounds is a very reliable and accurate spectroscopic technique to identifying the presence of different donor and acceptor functional groups in synthesized compounds. According to FT-IR spectra of Schiff base ligand, it is proved that the ligand showed keto- enol isomerism. In the keto form ligand coordinated with Cr³⁺ ion via azomethine nitrogen atoms. Due to the coordination behavior of ligand, the IR frequency of Cr (III) complex shifts from 1620 to 1680 cm⁻¹ after coordination of Cr (III) ion with Schiff base ligand which contains electron donor and electron acceptor functional groups.
**H¹ NMR Spectral Analysis of Ligand and Chromium (III) Complex**

The NMR shifts for the protons and carbon atoms of synthesized ligand and chromium (III) complex are shown in Fig.-3. The proton NMR spectra of the ligand and its chromium (III) complex can be classified into three distinct classes; the methyl (–CH₃) and methylene (–CH₂) protons appear as singlet peaks and resonate in the ranges 1.92–2.06 δ and 3.56–3.76 δ, respectively. The broad singlet peaks found between 3.86 and 4.74 δ are due to amine (–NH₂) protons and the peaks downfield in the region 6.58–7.96 δ which appear as multiplets are due to the aromatic protons. The chromium (III) complex with the methyl or methoxy group shows additional singlet peak due to methyl (–CH₃) protons at 2.28 δ or methoxy (–OCH₃) protons at 3.72 δ.

**Structural Studies**

The XRD technique is a very important crystallographic technique for the identification of the crystalline nature of materials synthesized via classical methods or modern methods like ultrasonication, biosynthesis and microwave-assisted synthesis. The structure and particles size of the synthesized compounds were characterized by a Bruker axis D8 phase X-ray diffractometer (XRD). X-ray diffractogram for Cr₂O₃ nanoparticles obtained from Cr(III) Complex of Schiff base ligand is shown in Fig.-4. The X-ray diffraction plots show peaks only due to Cr₂O₃ and no peak is detected due to any other material or phase indicating a high degree of purity of the as-synthesized sample. The broadening of the X-ray diffraction lines, as seen in Fig.-4 reflects the nanoparticle nature of the sample. In X-ray diffraction, some prominent peaks were considered and corresponding d-values were compared with the standard. X-ray diffraction shows that the formed nano metal oxide is pure Cr₂O₃ and has a hexagonal structure.

All peaks are matched with Cr₂O₃ nano structures JCPDS file number; 74- 0326 .card. Using Cu kα radiation (x = 1.540 A° in the 2θranging from 20 to 80°. According to JCPDS file number; 74- 0326 Cr₂O₃ nanoparticles in the hexagonal phase have been identified. Eight high-intensity diffraction at 2θ = 16.9°, 18.7°, 28.2°, 30.75°, 33.4°, 38.13°, 43.2°, and 48.1° from 111, 200, 311, 222, 400, 331, 420, 511 planes indicates the nanocrystalline nature of synthesized Cr₂O₃ nanoparticles. The average particle size of Cr₂O₃ nanoparticles was calculated by the Debye- Scherer’s Eqn.-1 is 48 nm.

\[
D = \frac{0.94 \lambda}{\beta \cos \theta}
\]  

(1)
Where D is the diameter of the nanoparticles λ is the X-ray wavelength is equal to 1.54 Å° and β is the half-width of the diffraction peak. Reflections of XRD Peaks are small in size and broad and indicate the nano size of Cr₂O₃ nanoparticles. Because the nanoparticles were synthesized through an aqueous extract of cinnamon bark are pure therefore no additional peaks are observed. Synthesis of the nano crystalline hexagonal shape of Cr₂O₃ nanoparticles may be due to the green synthesis of the formation of very small-sized Cr₂O₃ nanoparticles.

Fig.-3: H¹NMR Spectra of (a)Schiff Base Ligand and (c)Coordinated Cr (III) Complex

Table-1: Analytical and Physicochemical Data of Synthesized Compounds Using Glacial Acetic Acid As Catalyst

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds/Molecular Weight</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
<th>Elemental Analysis (Found/Calculated) %</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Schiff base ligand [C₅₈H₅₆O₁₀N₄] 968g/mol, (Orange color)</td>
<td>180-200°C</td>
<td>87%</td>
<td>C 46.60(46.70) H 4.20(4.30) N 11.35(11.40) Cr -</td>
<td>.52</td>
</tr>
<tr>
<td>2.</td>
<td>[Cr(C₅₈H₅₆O₁₀N₄ Cl₂].6H₂O 1138.5 g/mol, Brown Color</td>
<td>260-280°C</td>
<td>85%</td>
<td>C 49.91(47.87) H 4.03(3.97) N 10.14(10.10) Cr 7.36(7.32)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Morphological Studies
Transmission electron micrograph of Cr₂O₃ nanoparticles prepared from chromium (III) complex which was obtained by Schiff base ligand is shown in Fig.-5. Hexagonal shape nanoparticles are formed from the macro cyclic chromium (III) complex. The thickness and radius of nanoparticles are estimated using Gatan Software. Reduced FFT shown in Fig.-5 indicates high crystalline behavior of synthesized Cr₂O₃ nanoparticles and clear occurrence of lattice fringes in Cr₂O₃ nano particles. The presence of dark and bright fringes confirms the formation of the very good crystalline structure of nanoparticles.

TEM Morphological Studies
A transmission electron microscope is used to view a thin film section/specimen through which electrons can pass and generate a projection image. Figure-5 show the TEM images of ligands and their coordinated Chromium (III) complexes respectively. The uniformity and similarity between the particle forms of synthesized ligand and their coordinated Chromium (III) complex indicate that the existence of morphological phases has a homogenous matrix.

Thermal Behavior of Synthesized Compounds
The results of the thermal analysis for the coordination compounds in Table-2 and the thermograms are shown in the supplementary data shown in Figs.-6 and 7. The coordinated chromium(III) complex is stable.
at room temperature and did not change the color in safekeeping in a dry place. The thermal decomposition of the complex takes place in four steps. The first decomposition step, an endothermic one, in the temperature range of 800°C-1300°C is associated with all compounds to the loss of crystalline water. The second step of the thermal decomposition of the compounds corresponds to the elimination of coordinated water and takes place over a temperature range of 140°C-220°C, associated with an endothermic peak.

For the compounds second step can be correlated with the ligand side group releases, phenolic groups of ligand for complex, weight loss in accordance with the elimination of two water molecules. The next step of decomposition is a complex reaction step, being an overlap of process. The third stage of the thermal decomposition process corresponds to the loss of chloride ions. The last step of thermal decomposition was strongly exothermic corresponding to the oxidative degradation of the organic ligand residue. It starts from 380 to 4900°C and finishes around 750°C for all the compounds. The final residue of decomposition is CrO and the chromium percentage determine from this is in accordance with the theoretical content.

**In-vitro Antibacterial Activity of Synthesized Compounds**

The newly synthesized compounds were evaluated for their in-vitro antibacterial activity against three bacteria specially causing secondary infections in human beings viz *Escherichia coli, Bacillus subtilis* and *Pseudomonas aerugenosa*.
Table-2: Thermal Decomposition Data of Ligand and Coordinated Cr (III) Complex

<table>
<thead>
<tr>
<th>Complex</th>
<th>Step</th>
<th>Thermal Effect</th>
<th>Temperature Range (°C)</th>
<th>% Δm_{exp.}</th>
<th>%Δm_{calc.}</th>
<th>Chemical Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schiff base ligand [C₅₈H₅₆O₁₀N₄]</td>
<td>I</td>
<td>Endothermic</td>
<td>80-130°C</td>
<td>1.82</td>
<td>2.10</td>
<td>H₂O Loss</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Endothermic</td>
<td>110-210°C</td>
<td>3.4</td>
<td>4.32</td>
<td>2H₂O Coordinated Loss</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Exothermic</td>
<td>210-360°C</td>
<td>19.1</td>
<td>19.25</td>
<td>2HCl+2H₂O Loss</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Exothermic</td>
<td>360-780°C</td>
<td>65.00</td>
<td>65.66</td>
<td>Oxidative degradation of organic residue</td>
</tr>
<tr>
<td>[Cr(C₅₈H₅₆O₁₀N₄Cl₂)₂].6H₂O</td>
<td>I</td>
<td>Endothermic</td>
<td>60-80°C</td>
<td>1.94</td>
<td>2.20</td>
<td>H₂O Loss</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Endothermic</td>
<td>160-220°C</td>
<td>3.9</td>
<td>4.85</td>
<td>2H₂O Coordinate Loss</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Exothermic</td>
<td>230-490°C</td>
<td>22.4</td>
<td>24.2</td>
<td>Loss of SO₄²⁻ ions</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Exothermic</td>
<td>490-750°C</td>
<td>54.00</td>
<td>56.00</td>
<td>Oxidative degradation of organic residue</td>
</tr>
</tbody>
</table>

Zone of inhibition was measured in mm(Table-3). In this study, ampicillin drug used as standard antibiotic reference drug by known micro dilution broth susceptibility test method. The lowest concentration of compounds in µm/ml that prevent in-vitro growth of microorganism has been represented as MIC (minimum inhibitory concentration). Table-3. In the case of P. aerugenosa, compounds (MIC 30) showed antibacterial activity with a better zone of inhibition at concentrations (20, 40, 80 and 160µm/ml) in comparison to ligand and complex. No significant antibacterial activity was observed for electron donor and electron acceptor functional groups present in coordinated chromium (II) complex of Schiff base ligand.

Table-3: Zone of Inhibition and MIC Correlation Diagram of Schiff Base Ligand and their Coordinated Cr (III) Complex

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>E. coli Zone of Inhibition</th>
<th>B. sublilis Zone of Inhibition</th>
<th>P. aereugens Zone of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cinnamon bark</td>
<td>23mm</td>
<td>21mm</td>
<td>20mm</td>
</tr>
<tr>
<td>2</td>
<td>Schiff base ligand</td>
<td>25mm</td>
<td>30mm</td>
<td>26 mm</td>
</tr>
<tr>
<td>3</td>
<td>[Cr(C₅₈H₅₆O₁₀N₄Cl₂)₂].6H₂O</td>
<td>22mm</td>
<td>24mm</td>
<td>28mm</td>
</tr>
<tr>
<td>4</td>
<td>Cr₂O₃ Nanoparticles</td>
<td>25mm</td>
<td>20mm</td>
<td>27mm</td>
</tr>
</tbody>
</table>

**In-vitro Antioxidant Activity of Synthesized Compounds**

All the synthesized compounds were screened for antioxidant activity using DPPH assay. DPPH is a stable free radical compound and has been widely used to test the radical scavenging activity of numerous chemicals, including cinnamon bark and synthesized compounds. It is very good to note that DPPH activity of all the synthesized compounds is greater than cinnamon (standard), the results showed that compounds...
showed greater antioxidant activity than quercetin (positive control), and compound 3 showed greater antioxidant activity than ascorbic acid. The results of antioxidant activity of all the synthesized compounds data are in accordance with the theoretical aspects because the position and the number of the –OH groups as well as the degree of conjugation of the whole molecule are important. Antioxidant efficacy of natural flavonoids of similar conjugation level is roughly proportional to the presence of a total number of hydroxyl groups in the benzene ring.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>DPPH activity (IC$_{50}$ µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Schiff base ligand</td>
<td>1.74</td>
</tr>
<tr>
<td>2.</td>
<td>[Cr(C$<em>{34}$H$</em>{56}$O$<em>{10}$N$</em>{4}$Cl$<em>{2}$)$</em>{2}$H$_{2}$O]</td>
<td>2.72</td>
</tr>
<tr>
<td>3.</td>
<td>Cinnamon bark powder</td>
<td>1.01</td>
</tr>
<tr>
<td>4.</td>
<td>Quercetin</td>
<td>1.72</td>
</tr>
<tr>
<td>5.</td>
<td>Ascorbic Acid</td>
<td>3.76</td>
</tr>
</tbody>
</table>

TEAC- Trolox equivalent antioxidant capacity has been calculated from molar absorptive by dividing $1.64 \times 10^4$.

**Molecular Docking Analysis**

Current molecular docking studies revealed that Cr (III) complexes and Schiff base ligand both properly interacted with the target i.e. main protease (M$^{\text{pro}}$) of SARS-CoV-2. Recent findings suggested that this particular enzyme is made up of three domains having different amino acids residues vis. main viz., domain I (residues 8–101), domain II (residues 102–184) and domain III (residues 201–303) and as similar to other corona viruses, SARS-CoV-2 M$^{\text{pro}}$ also consist a Cys145-His41 catalytic dyad located in a cleft between domain I and domain II (Chhetri et al. 2021). Moreover, recently published literature suggested that three specific residues viz. His41, Cys145, and Glu166 are important for interactions and inhibition of this target enzyme. In this study, both synthetic ligands (Chromium (III) complex and acquired Schiff base ligand) could interact with an enzyme in domain I and domain II and could demonstrate significant binding affinity towards the target.

Although both molecules could not interact with above mentioned three amino acids which are supposed to be mandatory for inhibition of enzyme but other interactions afforded by both ligands could not be ignored as to date no specific inhibitors or other approaches found to be effective in the treatment of covid-19 and both molecules have shown potent antioxidant activity in intro conditions. We are emphasizing here antioxidant activity with the treatment of covid-19 because antioxidants are proven therapy in overcoming the symptoms of covid-19. This molecular docking study also concludes here that not only the active site but other sites in the target enzyme might be a reasonable area where interactions can be investigated for the development of better therapeutic agents for the treatment of covid-19.

Fig.-8: Structure (Chain A) of M$^{\text{pro}}$ of SARS-Cov-2 with domain I, II and III (Red circle represents the catalytically active site of M$^{\text{pro}}$).
In conclusion, the resulting synthesized compound of Schiff base ligand & coordinated chromium (III) complex were evaluated for their antimicrobial and antioxidant activity and compared with standard compounds. Synthesized Schiff base ligand, coordinated Cr (III) complexes showed more antimicrobial activity than standard compounds. The complex prepared from chromium chloride hexa hydrate salt showed maximum antioxidant activity, while antioxidant activity decreases in chromium oxide nanoparticles. All compounds are better scavengers of superoxide anions radical than Schiff base ligand. This scavenging activity is mainly due to the redox reaction within the Cr$^{3+}$/Cr couple and secondary to the phenolic functional group of the cinnamaldehyde derivative of Schiff base ligand. This research work also explains the fact that the chromium complex exhibits the highest antioxidant activity, this research work did not receive any specified amount from funding agencies in the public, commercial or non-profit section. Molecular docking studies of all the synthesized derivatives have been studied against the main protease (6LV7) of SARS - COV-2 protein. The docking result explained the various type of protein-ligand interaction and it is also seen that the ligands show significant interaction at the interface between domain...
I and domain II except for ligand. Furthermore, the compounds showed interaction at the Cystein 145 – His 41 catalytic dyad. The pharmacokinetic study (ADMET) has revealed that the ligand and coordinated chromium (III) complex could act as a potential drug candidate. The docking result explained the various type of protein-ligand interaction and it is also seen that the ligand show significant interaction at the interface between d. Furthermore, the compounds showed interaction with metal ions also in a very efficient way. Therefore we may conclude that the ligand and coordinated Chromium (III) complex could act as a potential antioxidant agent against M pro (6LU7) protease enzyme.

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REFERENCES


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