

***Ananas Comosus* Peel Extract Mediated Synthesis of Cobalt Oxide
Nanoparticles for Hexavalent Chromium Removal and Antibacterial Activity**



Abate Kassaw Shumete

A Thesis submitted to the Department of Applied Chemistry

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of

Master of Science in Applied Chemistry (Industrial Chemistry)

Office of Post Graduate Studies

Adama Science and Technology University (ASTU)

November, 2022

Adama, Ethiopia

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Co-Advisor: Eneyew Tilahun (M.Sc)

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DECLARATION

I declare that this Master thesis entitled “***Ananas Comosus* Peel Extract Mediated Synthesis of Cobalt Oxide Nanoparticles for Hexavalent Chromium Removal and Antibacterial Activity**” is my own original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other University. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled “**Ananas Comosus Peel Extract Mediated Synthesis of Cobalt Oxide Nanoparticles for Hexavalent Chromium Removal and Antibacterial Activity**” prepared under our guidance by Abate Kassaw Shumete submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry (Industrial Chemistry). Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

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APPROVAL PAGE

We, the advisors of the thesis entitled “**Ananas Comosus Peel Extract Mediated Synthesis of Cobalt Oxide Nanoparticles for Hexavalent Chromium Removal and Antibacterial Activity**” and developed by Abate Kassaw Shumete; hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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ACKNOWLEDGMENTS

First I would like to thank the almighty God who gave me the strength and wisdom to complete my work successfully. Then I would like to express my genuine heartfelt gratitude to my advisor, Dr. Bedasa Abdisa for his invaluable suggestion, comments, corrections, and professional and technical guidance throughout my work for the successful accomplishment of this thesis. I would like to express my deepest gratitude to my Co-adviser Mr. Eneyew Tilahun, for his useful suggestions, guidance, valuable comments, and friendly approach throughout this thesis work and contribution of ideas as well as sharing of his precious time to the work. I am deeply thankful to Dr. Fedlu Kedir and Dr. Dereje Tsegaye, for their supply of the priceless valuable chemical and the guidance that made this research possible on time. I would like to acknowledge my friend Terfo Yilma for his supported material, ideas and who encourages me to continue my study. I would like to express my thanks to the Department of Applied Chemistry, Adama Science and Technology University, for providing the necessary knowledge, assistance, and facilities to conduct my research work

TABLE OF CONTENT

Contents	Page
TABLE OF CONTENT	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS AND ACRONYMS	x
ABSTRACT.....	xi
1. INTRODUCTION	1
1.1. Background of Study.....	1
1.2. Statement of the Problem	3
1.3. Objectives of the Study	4
1.3.1. General Objective	4
1.3.2. Specific Objectives	4
1.4. Significance of the Study	5
1.5. Scope of the Study.....	5
2. LITERATURE REVIEW.....	6
2.1. Nanoparticles.....	6
2.2. Cobalt Oxide Nanoparticles	6
2.3. Synthesis Methods of Cobalt Oxide Nanoparticles.....	7
2.3.1. Physical Synthesis Methods of Cobalt Oxide Nanoparticles	8
2.3.2. Chemical Synthesis Methods of Cobalt Oxide Nanoparticles.....	8
2.3.3. Biological Synthesis Methods of Cobalt Oxide Nanoparticles	9
2.4. The Chemistry of <i>Ananas Comosus</i> Peel	11
2.5. Applications of Green synthesized Cobalt Oxide Nanoparticles	12
2.6. Water Pollution and Its Source.....	14
2.7. Toxic and Heavy Metal Ions	15

2.8. Wastewater Treatment Methods.....	17
2.9. Adsorption.....	18
2.9.1. Adsorption Isotherms	19
2.9.2. Adsorption Kinetic	20
2.10. Antibacterial Activity of Cobalt Oxide NPs	21
2.11. Antibacterial Strains.....	22
3. MATERIALS AND METHODS.....	23
3.1. Chemicals and Reagents.....	23
3.2. Apparatus and Instruments.....	23
3.3. Methods.....	23
3.3.1. Collection of <i>Ananas Comosus</i> Fruit Peel.....	23
3.3.2. Extract Preparation	24
3.3.3. Preliminary Phytochemical Screening of <i>Ananas Comosus</i> Fruit Peel.....	24
3.3.4. Synthesis of Cobalt Oxide Nanoparticles.....	25
3.4. Adsorption Study.....	26
3.4.1. Stock Solution Preparation	26
3.4.2. Batch Adsorption Experiment	26
3.4.3. Optimization Experiment	27
3.4.4. Adsorption Isotherm.....	27
3.4.5. Adsorption Kinetics.....	27
3.5. Antibacterial Activity of Cobalt oxide Nanoparticles.....	27
3.5.1. Media Preparation and Bacterial Culture	28
3.5.2. Disc Diffusion Method	28
4. RESULTS AND DISCUSSIONS.....	29
4.1. Phytochemical Analysis of the Peel Extract	29
4.2. Characterization of Cobalt Oxide Nanoparticles	30

4.2.1. Thermal Gravimetric-Differential Thermal Analysis (TGA-DTA).	30
4.2.2. X-Ray diffractometer (XRD) Analysis of Calcinated Co_3O_4 NPs	31
4.2.3 Scanning Electron Microscopic (SEM) Analysis	33
4.2.4. Energy Dispersive X-Ray Spectroscopy (EDS) Analysis	34
4.2.4. Ultraviolet-visible Diffuse Reflectance Spectroscopy (UV-Vis DRS) Analysis	35
4.2.5. Fourier Transform Infrared (FTIR) Analysis.	36
4.2.6. Brunauer-Emmett-Teller (BET) Analysis	37
4.3. Adsorption Experiment	39
4.4. Optimization of Parameter	41
4.4.1. Effect of pH	41
4.4.2. Adsorbent Dosage.....	42
4.4.3. Effect of Initial Concentration of adsorbate	43
4.4.4. Effect of Contact Time	44
4.5. Adsorption Isotherms	45
4.5.1. Langmuir isotherm model	45
4.5.2. Freundlich isothermal model	47
4.6. Kinetic Study.....	48
4.6.1. Pseudo First Order Kinetics Model	49
4.6.2. Pseudo Second-Order Kinetic Model	49
4.7. Antibacterial Activity of Cobalt Oxide Nano-drug.....	51
5. CONCLUSION AND RECOMMENDATIONS	54
5.1. Conclusion.....	54
5.2. Recommendations	55
REFERENCES	56

LIST OF TABLE

Tables'	page
Table 1: Green Co ₃ O ₄ NPs synthesis with numerous templates and there applications.....	11
Table 2: Various conventional methods of wastewater treatment techniques	18
Table 3: Phytochemical screening of ananas comosus peel extract	30
Table 4: Calculated crystal size of synthesized Co ₃ O ₄ NPs in three volume ratios.	33
Table 5: Surface area and pore size of synthesizedCo ₃ O ₄ NPs by (BET) (BJH) analysis,.....	39
Table 6: The removal efficiency and adsorption capacity of Co ₃ O ₄ NPs	40
Table 7: Langmuir adsorption isotherm parameter.....	47
Table 8: Freundlich Adsorption Isotherm parameters	48
Table 9: Linear equations of the pseudo-first and pseudo-second order equation	51
Table 10: Zone of inhibition of Co ₃ O ₄ nano-drug against <i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. pyogen</i>	52

LIST OF FIGURE

Figures	page
Figure 1: Some selected types of plants used as template to synthesized Co_3O_4 NPs.....	10
Figure 2: Main sources of heavy metal ions	15
Figure 3: Diagrammatic representation of synthesized cobalt oxide nanoparticles.	26
Figure 4: The color change observed when the peel extract of <i>Ananas comosus</i> was tested for the presence (ii) Reducing sugar,(iii) Phenol (iv) Alkaloids, (v), Flavonoids, (vi) Saponins (vii) Terpenoids (viii) Tannins.	29
Figure 5: TGA-DTA graphs of Uncalcinated Co_3O_4 (1:1) NPs.	31
Figure 6: XRD pattern of <i>Ananas comosus</i> peel extract synthesized Co_3O_4 NPs.	32
Figure 7: The SEM image of Co_3O_4 NPs a (1:1), b (1:2) and c (2:1) volume ratios.	34
Figure 8: EDS image of Co_3O_4 (1:1) NPs, a (SEM image of target sample), b (image of Co_3O_4 NPs composition) c (EDS image Co_3O_4 NPs).	35
Figure 9(a): UV-visible reflectance spectra and (b), band gap energy of Co_3O_4 NPs.....	36
Figure 10: FTIR spectra of (a) <i>Ananas comosus</i> peel powder extract (b) uncalcinated synthesized of Co_3O_4 NPs and (c) calcinated synthesis of Co_3O_4 NPs.....	37
Figure 11: BET surface area and BJH pore size analysis of Co_3O_4 NPs.	38
Figure 12: Calibration graph of prepared standard solution.	39
Figure 13: The removal efficiency of different volume ratios of Co_3O_4 NPs.	41
Figure 14: Effect of pH on the adsorption of Cr (VI).	42
Figure 15: Effect of adsorbent dosage of Co_3O_4 NPs for adsorption Cr (VI).	43
Figure 16: Effect of initial concentration on adsorption of Cr (VI).	44
Figure 17: Effect of contact time on the adsorption of Cr (VI).	45
Figure 18: Langmuir adsorption isotherm model.	46
Figure 19: Freundlich Adsorption Isotherm.	48
Figure 20: Pseudo first-order kinetic curve.	49
Figure 21: Pseudo second order kinetics curve.....	50
Figure 22: The zone of inhibition of synthesized Co_3O_4 (1:1, 1:2, and 2:1 ratio) nano-drugs against <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>S. pyogenes</i> at 50, 75, and 100 $\mu\text{g/mL}$ concentration.	51

LIST OF ABBREVIATIONS AND ACRONYMS

AP	Ananas peel
BET	Brunauer Emmett Teller
BJH	Barrett-Joyner Halenda
CANPs	Cationic Nanoparticles
CFU	Colene Forming Unit
Co-MPA	Cobalt-Mercaptopropionic Acid
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
DRS	Diffraction Reflectance Spectroscopy
DTA	Differential Thermal Analysis
<i>E.Coli</i>	<i>Escherichia Coli</i>
EDX	Energy-Dispersive X-ray spectroscopy
FT-IR	Fourier Transform Infrared Spectrophotometry
HMI	Heavy Metal Ions
MRI	Magnetic Resonance Image
NPs	Nanoparticles
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PVD	Physical Vapor Deposition
RO	Reverse Osmosis
ROS	Reactive Oxygen Species
<i>S.aureus</i>	<i>Staphylococcus aureus</i>
<i>S. pyogen</i>	<i>Streptococcus pyogen</i>
SEM	Scanning Electron microscope
TGA	Thermal Gravimetric Analysis
t/ha	tone per hectare
UV-visible	Ultra Violet Visible Spectroscopy
WHO	World Health Organization
XRD	X-ray Diffractometer
ZOI	Zone of Inhibition

ABSTRACT

Hexavalent chromium ion from different sources is the most serious water pollutant, which causes carcinogenic and related problems to humans and animals. In addition, the development of antibiotic-resistant bacteria is one of the most serious problems in health centers, and so requires new drug development to mitigate the problem. The focus of this research was the biosynthesis of Co_3O_4 NPs to address the problems as an adsorbent and a nano-drug. Co_3O_4 NPs were synthesized via a biological method using *Ananas comosus* peel extract as a template in the presence of 0.5M of cobalt nitrate hexahydrate in different volume ratios such as 1:1 (50 mL of precursor salt to 50 mL of extract), 1:2 (33.3 mL of precursor salt to 66.7 mL of extract), and 2:1 (66.7 mL of precursor salt to 33.3 mL of extract). The synthesized Co_3O_4 NPs were characterized using TGA-DTA, XRD, SEM-EDS, UV-Vis DRS, BET, and FTIR. TGA-DTA analysis showed that the biosynthesized Co_3O_4 was stable above 500°C and SEM analysis result showed spherical shaped Co_3O_4 NPs. From the XRD result, the average crystallite size was found to be 12.58, 13.40, and 22.05 nm for 1:1, 1:2, and 2:1 ratios, respectively. The chemical composition and morphology of Co_3O_4 NPs was studied by SEM-EDS analysis. The energy band gap and specific surface area of Co_3O_4 NPs of 1:1, 1:2, and 2:1 volume ratios were estimated to be 3.33, 2.95, 2.70 eV with UV-Vis DRS, and 24.7, 23.7, and 22.5 m^2/g with BET analysis respectively. FTIR analysis confirmed the presence of bioactive compounds in peel extract and indicated the formation of Co-O bond stretching. The 1:1 volume ratio showed enhanced adsorption efficiency due to small average crystal size and high surface area to volume ratio and, selected for adsorption of Cr (VI) ions in aqueous solution. After optimization of each parameter the maximum adsorption efficiency of Cr (VI) was achieved to be 95% using 1g adsorbent, at 2pH, 20 mg/L initial concentration and 120 min contact time at room temperature. The adsorption isotherm model was found to be best fitted to Freundlich's isothermal model ($R^2=0.999$) and the adsorption kinetics was found to fit pseudo 2nd order kinetic model with $R^2=0.994$ in presence of Co_3O_4 (1:1) NPs. In addition the antibacterial activity of Co_3O_4 nano-drug synthesized using 1:1, 1:2, and 2:1 ratios was investigated and was found in the range of 8-11, 9-13, and 7-10 mm inhibition zone, respectively against *E.coli*, *S.aureus*, *P.aeruginosa*, and *S. pyogen*, which was done using disc diffusion method.

Keyword: Adsorption, Co_3O_4 NPs, Biosynthesis, Antibacterial activity.

1. INTRODUCTION

1.1. Background of Study

Water pollution is the most paramount issue of the world, especially toxic heavy metal ions' is one of the most severe environmental challenges that threaten human beings all over the world (Saeed et al., 2020). Heavy metal ions are released from different sources (Qu et al., 2020). It can be accumulated through food chain organisms and have serious impacts on the environmental ecosystem and human health by the physicochemical properties of highly virulent (killer), hardly degraded, and widely dispersed in water. Some elements like Mn, Fe, Mo Cr, Zn, Se, Co, Cu, and Ni are important as micronutrients, however, they are potentially toxic elements, when they exceed optimum concentrations, in that they may interfere with or inhibit the actions of cellular enzymes. Hg, Cd, Pb, and As have no biological function and are toxic to all living organisms, and generally the main toxic heavy metal contaminants are Pb^{2+} , Cd^{2+} , Cr^{6+} , Ni^{2+} , Zn^{2+} , Cu^{2+} , and Hg^{2+} ions (Singh et al., 2018). Among these, chromium ions are a potentially toxic and carcinogenic metal originating from natural processes and anthropogenic activities such as iron steel, electroplating, leather industries, and weathering of rocks (Qu et al., 2020). However, trivalent chromium is present in water, and food in trace volume seems benign while; hexavalent chromium is mutagenic; not essential for mammals, and has no biological confirmation (Samuel et al., 2021).

The wide range of pH Cr^{+6} oxyanions are soluble in water and can easily accumulate into underground water and pollute the consumption of water, and can cause significant alterations to the biochemical cycles of living things, and also, Cr^{6+} is a substantial metal that is broadly utilized in industries e.g electroplating, stainless steel production, leather tanning, textile manufacturing, fertilizer and wood preservation (Rhaman & Karim, 2020). Several treatment methods for the removal of chromium ions from aqueous solutions have been reported, mainly such as physical, chemical, and biological methods. However, these methods have a drawback such as using high cost of chemicals, increased concentration of dissolved solids in the effluent, high startup costs, and low efficiency to remove metal ions (Wadhawan et al., 2020). Among, these methods adsorption is the most useful for chromium removal and has significant advantages such as low cost availability, profitability, ease of operation, and high adsorption efficiency as compared to other methods (Hyder et al., 2015). Several studies are focusing on more innovative and effective materials as adsorbents. Various adsorbents such as clays (Bujdák, 2020) , bone char (Hyder et al., 2015), and chitosan (Kiani et al., 2021), have been used as adsorbents. However, high chemical and energy

requirements, generation of toxic by-products, low removal efficiency for low Cr concentration, production of large chemical wastes, and incomplete removal limit the wide applicability of these treatment techniques (Wadhawan et al., 2020). The biosorption process has been considered a highly effective and economical alternative (Peng & Guo, 2020). In addition to this different developing adsorbent systems, the design and fabrication of nanoparticle-based adsorbents have generated great interest in a variety of scientific communities ranging from chemical, biological, and environmental science (Saeed et al., 2020). In addition to water contamination, the increasing of antimicrobial resistance bacteria's is the most serious problem in the world. Some pathogens which were curable in past now are becoming untreatable such as Methicillin-resistant *staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* and that affects human health (Raja et al., 2018). The most commonly available antibacterial agents in the medical community are antibiotics. So, the development of antibiotic-resistant bacteria as a result of the broad use and abuse is becoming a major problem and lead to infectious diseases that were under control for years. Today, materials in nano size are one of the novel sources of antibacterial material (Al-fakeh, 2021).

Metals-based NPs and investigation of their antimicrobial activity could be one of such alternatives. Because of their unique characteristics such as size distribution and morphology, nanoparticles exhibit completely new properties (Al-fakeh, 2021). The nanoscaled materials have been attracted to numerous applications to remediate inorganic and organic pollutants from the environment and wastewater and also used to synthesis new drugs (Waris et al., 2021). Recently cobalt oxide nanoparticles have received great attentions from the researchers because of its unique applications in various sectors such as multifunctional p-type semiconductor photocatalytic, antimicrobial activity, and low cost as compared to precious metals like Pt, Pd, and Au (Al-fakeh, 2021). Several synthesis methods such as physical, biological, and chemical methodologies have been developed to synthesize nano crystalline Co_3O_4 NPs. Among these, the green synthesis method (plant-mediated synthesis of nanoparticles) is established as an alternative due to simplicity, a rapid process that involves the use of non-toxic and environmentally-friendly materials as compared to conventional techniques on the other hand, Plants and their extracts contains biologically active compounds that ability to donate hydrogen and quench oxygen which results in the reduction of metal ions to metal NPs (Mohammadi et al., 2021). Pineapple (*Ananas comosus*) is a tropical plant and fruit belonging to the family of Bromeliaceae, named after the enzyme bromelain, which is one of its most important health-promoting compounds. *A. comosus* is known as the fruit of kings, despite the fact that the fruit had become familiar to almost the whole world. *Comosus* exhibits antibacterial,

antiviral, antifungal, antiparasitic, and anti-inflammatory properties and *Comosus* peel have varying degrees of antimicrobial activity against *E. coli*, *S. aureus*, *K. pneumonia*, and *B. cereus* (Dabesor & Umoiette, 2018). *Ananas comosus peel* (AP) has significant medicinal importance and contains phytochemicals suitable for the efficient synthesis of nanoparticles (Akpomie et al., 2021). Therefore, this study aimed to synthesize cobalt oxide nanoparticles using *Ananas comosus* peel extract as a reducing and stabilizing agent, to characterize the synthesized nanoparticles using TGA-DTA, XRD, SEM-EDS, UV-DRS, BET UV-Visible and FTIR techniques and evaluate their application for removal of hexavalent chromium from an aqueous solution, and antibacterial activity against selected drug-resistant bacteria stains.

1.2. Statement of the Problem

Wastewater from textile mills, tanneries, electroplating, galvanizing, metallurgical industries, paint industries, and refining operations contain significant amounts of toxic heavy metal ions such as Hg^{2+} , Pb^{2+} , Cd^{2+} and Cr^{6+} . Among those Cr^{6+} has a potential risk to the whole environment in general and water pollution in particular, has raised severe environmental impacts because of its non-biodegradability, carcinogenicity, persistence, bioaccumulation nature to be accumulated in living tissues, causing various diseases and disorders. Their toxicity can result in reduced mental and central nervous functions, lower energy levels, and damage to the blood composition, lungs, kidneys, liver, and other vital organs. Among these Cr^{6+} ions is very toxic and cause for the above mentioned diseases. It is important to remove from wastewater before it's disposed to the aquatic environments by using effective methods. However, various physicochemical remediation technologies have been developed for the removal of Cr^{6+} ions from aqueous solution such as chemical precipitation, filtration, RO, solvent extraction, ion exchange, coagulation and adsorption techniques. Those methods have disadvantages such as high operation cost, production of a high amount of sludge, use of additional pretreatment and low removal efficiency. However, from these methods, adsorption seems to be the most one because of its simple operation, cost effectively, environmental friendly, better remediation sensitivity, higher uptake capability, efficiency to remove mini/little adsorbate in solution, used easily available materials as adsorbent and using low-cost nanosized adsorbents. Then small size Co_3O_4 NPs is of great interest due to its distinctive physiochemical properties such as it provides high surface area, catalytic, p-type semiconductor, magnetic properties and cost-effectiveness (Al-fakeh, 2021).

The emergence of infectious diseases, in general, poses a serious threat to public health worldwide, especially with the emergence of antibiotic-resistant bacterial strains which extend to economic and social complications. Generally, both Gram-positive and Gram-negative bacterial strains are thought to present a major public health problem due to the lack of suitable vaccines. Many commercial antibiotics are available in the world for the treatment of bacterial infectious diseases. However, the most problems with infectious diseases are bacterial infection not only its mortality and morbidity but also in terms of treatment costs, low efficiency of treatment, and insufficient distribution to society. A possible alternative is the synthesis of new inorganic compounds with antimicrobial applications. Over the years, environmental antibiotics have been used to control infections resulting from both community and hospital environments. For this reason in underdeveloped and developing countries like Ethiopia, those microbial infection diseases were serious health hazards, especially for children and old aged people due to the lack of suitable vaccines. Therefore, the present work aimed to synthesize Co_3O_4 NPs from its salt precursor, and *Ananas comosus* peel extract, and evaluate its activity as adsorbents for removal chromium (IV) ions in the aqueous solution and antibacterial activity against some selected bacteria strains.

1.3. Objectives of the Study

1.3.1. General Objective

General objective of this study was synthesize a low cost and effective Co_3O_4 nano adsorbent for the removal of chromium (VI) ion from an aqueous solution and to investigate antibacterial activity against selected drug-resistant bacteria strains.

1.3.2. Specific Objectives

- To synthesize Co_3O_4 NPs using *Ananas comosus* fruit peel extract
- To characterize the synthesized cobalt oxide NPs using TGA-DTA, XRD, BET, SEM-EDS, UV-DRS, and FTIR spectroscopic techniques.
- To investigate the adsorption efficiency of synthesized Co_3O_4 nano-adsorbent for hexavalent chromium ions removal from an aqueous solution.
- To evaluate antibacterial activity of synthesized Co_3O_4 NPs against *Streptococcus pyogenes*, *Staphylococcus aureus* *Escherichia coli* and, *Pseudomonas aeruginosa*

1.4. Significance of the Study

The study demonstrated the value of biogenic synthesis methods and the function of biocompatible green materials in technologically and financially viable procedures and practices of *Ananas comosus* peel. It also summarizes the quest for an environmentally sustainable synthesis process of Co_3O_4 NPs using *Ananas comosus* peel extract for their application to the field of environmental sustainability like removal of heavy metal ions from aqueous solutions and their antibacterial activity. All communities, scientific communities, companies, and up-and-coming researchers will profit from the study's findings as nano adsorbents and nano-drugs. This study makes it possible to conduct additional research on the local use of *Ananas comosus*.

1.5. Scope of the Study

The scope of the study was limited to test phytochemicals constituents in *Ananas comosus* peel extract used as reducing or capping agent and to synthesized Co_3O_4 NPs using cobalt nitrate hexahydrate salt and *Ananas comosus* peel extract; characterized the synthesized Co_3O_4 NPs using TGA-DTA, UV-DRS, XRD, SEM-EDS, BET, and FT-IR. And also, the results were also specific to evaluate the adsorption efficiency and adsorption capacity of synthesized Co_3O_4 nano adsorbent for the removal of Cr (VI) from aqueous solution, and to investigate antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and, *Pseudomonas aeruginosa*.

2. LITERATURE REVIEW

2.1. Nanoparticles

Nanoparticles (NPs) are a wide class of materials that include particulate substances, which have one dimension less than 100 nm at least. Depending on the overall shape these materials can be 0D, 1D, 2D, or 3D. NPs is not simple molecule themselves and are therefore composed of three layers i.e. (a) the surface layer, which may be functionalized with a variety of small molecules, metal ion, surfactants, and polymers. (b) The shell layer, which is a chemically different material from the core in all aspects, and (c) The core, which is essentially the central portion of the NPs and usually refers to the NPs (Khan et al., 2019). The unusual properties of nanoparticles such as hardness, rigidity, high yield strength, flexibility, and ductility, are attributable to one of the most critical characteristics of nanoparticles which is the high surface-to-volume ratio (Akshatha et al., 2021). Some physicochemical properties of nanoparticles can be altered with their varying size. For instance, by decreasing the size, particles change color, semiconducting materials exhibit metallic properties, and nonmagnetic particles become magnetic (Dubey et al., 2018). When the particle dimensions of semiconductor materials are smaller increases the energy bandgap. Nanoparticles that possess magnetic properties offer exciting new opportunities including improving the quality of magnetic resonance imaging (MRI), hyperthermic treatment for malignant cells, site-specific drug delivery, food industry, catalysis, medical, and also the recent research interest in manipulating cell membranes (Khan et al., 2019).

2.2. Cobalt Oxide Nanoparticles

Metal oxide nanoparticles (MONPs) exhibit special chemical and physical properties due to their fixed size and more density of the corner (Al-fakeh, 2021). Some metal oxide nanoparticles, for example, iron oxides, cobalt oxide, zinc oxide, and copper oxide have various methods that can be synthesized and are very important for various applications (Haq et al., 2021). Among the metal oxide nanoparticles cobalt oxide nanoparticles has attracted a lot of attention in the scientific community due to their ecological nature, ease of use, low cost, strong electrical potential, and wide applications such as gas sensors, supercapacitors, antiferromagnetic, multifunctional p-type semiconductor, heterogeneous catalysis, and magnetic semiconductors (Dewi et al., 2019).

Cobalt in its oxide form with variable oxidation state is an excellent photocatalyst; it is cheap too, hence used to eliminate toxic chemicals and dyes from contaminated water (Waris et al., 2021).

Co_3O_4 NPs is a form of spinel compound with mixed valent, Co-O system, with cubic close packing structure in which Co (II) and Co (III) ions occupies tetrahedral 8a and octahedral 16d positions. Tricobalt tetraoxide (Co_3O_4) is the material of most interest in the field of catalysis, supercapacitors, energy storage, and electrochemical sensors (Dubey et al., 2018). Also, cobalt oxide nanoparticles have various biomedical applications because of their distinctive antioxidant, antimicrobial, antifungal, anticancer, larvicidal, antileishmanial, anticholinergic, wound healing, and antidiabetic properties. Co_3O_4 NPs is suitable for use in electronic devices and superconducting materials because having corrosion and oxidation resistance (Waris et al., 2021). Generally, Co_3O_4 NPs is available in three forms, cobaltic oxide (Co_2O_3), cobaltous oxide (CoO), and cobaltous oxide (Co_3O_4). The direct optical band gaps of Co_3O_4 nanoparticles were about 1.48 and 2.19 eV and it can be used as a photocatalyst to degrade organic pollutants using visible light (Dewi et al., 2019). Co_3O_4 NPs is synthesized by physical, chemical, and biological methods. However, the biological synthesis approach is eco-friendly, simple, reliable, biocompatible, and easy for the synthesis of NPs (Waris et al., 2021).

2.3. Synthesis Methods of Cobalt Oxide Nanoparticles

Various physicochemical-based methods have recently been utilized to synthesize different types of Co_3O_4 NPs but top-down and bottom-up are the most common approaches (Waris et al., 2021). In the physical (top-down) method, the rupture of bulk materials to fine particles is conducted by various techniques such as evaporation, condensation, laser ablation, or other physical methods. These methods need more energy and high cost. However, in the bottom-up method, the atoms are assembled into nuclei and then grown into NPs. Biological and chemical methods which are used for the synthesis NPs are considered bottom-up approaches (Shafiq et al., 2021). Whereas the bottom-up is a building approach that involves the assembly of atomic size to form nano-sized particles. The bottom-up approach is further divided into two classes, biological and non-biological approaches. Non-biological approaches include template support synthesis, flame spraying, spinning, laser pyrolysis, atomic condensation, and deposition of chemical vapors (Shafiq et al., 2021). These methods also use toxic chemicals, which are expensive and time-consuming, whereas the biological approach uses various biotic resources such as plants, algae, microorganisms, and other biological molecules like starch, egg albumin, and gelatin for the production of different types of NPs. This biological approach is also known as the green approach. This method is ecofriendly, simple, reliable, biocompatible, and easy for the synthesis of NPs (Waris et al., 2021).

2.3.1. Physical Synthesis Methods of Cobalt Oxide Nanoparticles

Physical synthetic methods are one of the syntheses techniques of cobalt oxide nanoparticles. The physical method often called the top-down approach which includes methods like Laser ablation, physical vapor deposition, thermal evaporation, thermal decomposition, etc (Prabaharan et al., 2017). Physical vapor deposition (PVD) has been used to produce Co_3O_4 nanowires with very good repeatability. This process consists of the evaporation of the precursor (metal oxide), the subsequent transport, and the final condensation onto the substrate. It is a relatively simple process and the deposition is controlled by temperature, pressure, and carrier gas flow (Mehl et al., 2016). [Bis(salicylidene) cobalt (II)] was used as a precursor to prepare cobalt oxide nanoparticles of an average diameter of 25-35 nm by thermal decomposition method (Salavati-Niasari et al., 2008). Thermal evaporation is a technique, and a simple process in which condensed or powder source material is vaporized at elevated temperature and the resultant vapor phase condenses under a certain condition (pressure, temperature, substrate, etc.) to form desirable products (Abdel-Aziz et al., 2020). The other physical methods are the Pulsed laser ablation (PLA) method is cheap, easy, and we will not have any byproducts after production. Laser ablation is a physical, environment friendly method, and can be introduced as an efficient approach to prepare a variety of nanoscale materials. Interaction of laser light with metal nanoparticles happens via its absorption by free electrons that exhibit Plasmon resonance, the position of which is determined by the concentration and particle size (Ghaem et al., 2019). Generally, physical methods are needed more energy and high cost and raise many environmental concerns, expensive and cumbersome (difficult) for the large-scale production of nanoparticles.

2.3.2. Chemical Synthesis Methods of Cobalt Oxide Nanoparticles

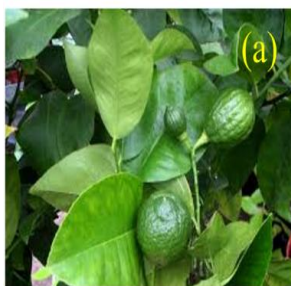
Several methods such as Co-Precipitation, hydrothermal, microemulsion and son-chemical methods, sol-gel combustion, wet polymerization, solvothermal methods, and hydrothermal methods are types of chemical methods that are used to synthesize cobalt oxide nanoparticles in different sizes, shapes, and morphological structures (Iravani & Varma, 2020). Cobalt oxide (Co_3O_4) NPs were synthesized from cobalt nitrate and ammonium oxalate using the precipitation method the average particle size of Co_3O_4 NPs was found to be 52 nm by the TEM and SAED studies and the shape was spherical- established by the SEM analysis (Thambidurai et al., 2021).

And, cobalt oxide (Co_3O_4) nanorods were prepared by a simple and new sol-gel synthesis method using ethanol solution of cobalt nitrate as a precursor and Cetyl trimethyl ammonium bromide

(CTAB) as surfactant. Morphological properties of the nanoparticles were characterized with in SEM. The SEM images revealed that the particles changed from spherical shape to rod-like shape by increasing annealing temperature and the size of the particle decreased from 25nm to 50nm with an increased temperature (Shadrokh et al., 2016). The other chemical synthesis methods of Co_3O_4 NPs are achieved by hydrothermal synthesis using Co-MPA (3-Mercaptopropionic acid) within 10min. The shape of Co_3O_4 depends upon temperature. While the low temperature favors spherical-shaped nanoparticles, the high temperature favors cubic shape 12-20 nm size particles (Khadar et al., 2018). Usually, chemical methods take a long time, and we will have different kinds of toxic and chemical agent byproducts after the production process

2.3.3. Biological Synthesis Methods of Cobalt Oxide Nanoparticles

The green synthesis approaches are gaining significant acceptance from the scientific community. Currently, the synthesis of biogenic NPs has attracted a great deal of attention for their cost-effectiveness, environmental benignity, and relative ease of synthesis (Kamran et al., 2019). Co_3O_4 NPs was synthesized from an aqueous extract of red algae and the average crystal size was 28.2 ± 7.8 nm based on the X-ray diffraction analysis and uniform spherical morphology with an average diameter of 29.8 ± 8.6 nm according to TEM and SEM images and their utilization as cytotoxicity, antioxidant, anticoagulant, antibacterial, and anti-cancer properties (Ajarem et al., 2021). Co_3O_4 -NPs was biologically synthesized using the *Jumbo Muscatine (Vitis rotundifolia)* plant extract in average particle size of 40 nm is formed when pH is 8 to 9, based on XRD and the morphologies of Co_3O_4 show irregular nanotubes, 45 nm in size, according to SEM using $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ as cobalt salt (Samuel et al., 2020). Co_3O_4 NPs were fabricated using *Punica granatum peel* extract from cobalt nitrate hexahydrate at low temperatures. The synthesized cobalt oxide size was in the range of 40-80 nm and it utilized photocatalytic activity for the degradation of methyl orange color (Bibi et al., 2017). Green synthesis of cobalt oxide is the technique in which metal salts like $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Co}(\text{OH})_2$, and CoCl_2 mix with different plant parts it gives cobalt oxide nanoparticles, and the hydrothermal temperature are the primary factors controlling the morphology of cobalt oxide nanoparticles (Waris et al., 2021). Figure1 and Table 1 shows various plant parts and microbes that used for synthesized of Co_3O_4 NPs.



Citrus medica



Clover of Allium Sativum



Calotropis



Sageretia thea



Punica granatum



Eichhornia crassipes



Aspalathus linearis



Solanum tuberosum

Figure 1: Some selected types of plants used as template to synthesized Co₃O₄ NPs.

Table 1: Green Co₃O₄ NPs synthesis with numerous templates and there applications

Template	Size and shape	Function	ZOI& % of degradation efficiency	References
Piper nigrum (P. nigrum)leaves extract	29.10 nm spherical	Antibacterial activity	<i>S.aureus</i> 18.3mm <i>E.coli</i> 8.4mm	(Haq et al., 2021)
Sageretia thea	20.03 nm cubic	Antibacterial activities	<i>S. aureus</i> 26 ± 6.2 <i>E.coli</i> 20.2 ± 3.4mm	(Waris et al., 2021)
Populus Ciliata leaf	15-35 nm	Antibacterial activities	<i>E. coli</i> 6.8mm <i>B. subtilis</i> 13.7mm	(Hafeez et al., 2020)
Jumbo Muscadine (Vitis rotundifolia)	20-60nm spherical	The, photocatalytic activity of acid Blue-74	98%of-removal efficiency at t=150min at pH =10& 60mg/mL AB-74	(Samuel et al., 2020)
Red algae	29.8 ± 8.6 nm by SEM&28.2 ±7.8nm in XRD	Antibacterial anticancer, antioxidant	<i>S.aureus</i> 29.2 ± 8.7 <i>Bacillus subtilis</i> 28.6 <i>E.coli</i> 22.8 ± 6.5mm	(Ajarem et al., 2021)
Aspergillus nidulans fungi	20.29 spherical	Antifungal activity	Good antibacterial and antifungal activity	(Waris et al., 2021)
Aspergillus fbrasiliensis fungi	20-27nm Quasi-spherical	Antioxidant activity	Highest, DPPH radical scavenging (57%)	(Waris et al., 2021)
Turbinaria confides (alge)	Spherical,96 nm	Biomedical Applications	Antibacterial and Anticancer	(Shafiq et al., 2021)
Hibiscus, Rosa-Sanenness Flower	40.05-61.37 nm Regular	antibacterial activity	<i>Bacillus subtilis</i> 18.3 <i>E. Coli</i> 10.2 mm	(Iravani & Varma, 2020)

2.4. The Chemistry of *Ananas Comosus* Peel

Pineapple (*Ananas comosus*) is the leading edible member of the Bromeliaceae family. In terms of its importance in global production, it ranks second among four major fresh tropical fruits, where it comes after mango and is followed by papaya and avocado (Das et al., 2019). It is reported to have a sweeter taste, cylindrical shape with solid golden-yellow pulp, a very pleasant aroma when ripe, low acidity and longer shelf life compared to other varieties. It is also a good source of health-promoting antioxidants such as ascorbic acid, β -carotenes, and phenolic compounds. The fruits were harvested when they were one-third ripe (about 120 days after flowering was successfully induced (Dabesor & Umoiette, 2018).

Pineapples may be cultivated from a crown cutting of the fruit, possibly flowering in 20-24 months and fruiting in the following six months. *Ananas comosus* peels, core extracts, and crown extract contain sugar especially fructose, sucrose, and glucose (Mulualet al., 2021). The main volatile compounds found in pineapple pulp and cores are esters, followed by terpenes, ketones, and aldehydes biomolecules with carbonyl, hydroxyl, and amine functional groups have the potential for metal ion reduction and capping the newly formed particles during their growth processes (Akpomie et al., 2021). ZnO NPs were successfully synthesized from pineapple peel extract at 60°C and 28°C which acted as a natural reducing agent in the bio reduction process at 60°C resulting in a mixture of spherical- and rod-shaped structures and the smallest ZnO NPs (Akpomie et al., 2021).

Ag NPs were found to be effectively biosynthesized using the outer peel extract of the *Ananas comosus* fruit. The higher content of flavonoids and phenolic acids existing in the *Ananas comosus peel* extract could have supported the fast reduction of Ag^+ ions and better stabilization agent of Ag NPs. exhibited high anti-diabetic potential with higher cytotoxic activity against the cancer cell lines in a dose-dependent manner. It also exhibited moderate antioxidant and antibacterial activity (Das et al., 2019). In Ethiopia, pineapple is first introduced in the 1940s by a religious church in Sidama and Gedeo zones of southern region. Currently, the crop is cultivated by small-scale farming mainly in South and South-Western parts of the country and the average yield of the crop is below 50 tons/ha as compared to the global average fruit yield of 63 t/ha. The crop is mainly grown in Ethiopia to ensure food and nutrition security (Mulualet al., 2021). One of the options is to collect the peel of the disposed of pineapple and explore its utility, particularly as a sorbent for pollutant removal. This waste peel being available at no cost will help to reduce the cost of wastewater treatment in an attempt towards transforming waste into a resource and provide an alternative to the existing commercial available adsorbents. In this direction, the main purposes of the present study have been to evaluate the efficiency of Co_3O_4 NPs synthesized from *ananas comosus peel* to remove Cr(VI) from aqueous solution at different operational conditions and to test their antibacterial activity.

2.5. Applications of Green synthesized Cobalt Oxide Nanoparticles

Cobalt and cobalt oxide NPs are gaining diverse catalytic and biomedical applications, because of their unique antimicrobial, anticancer, catalytic, antioxidant, antifungal, and enzyme inhibition properties (Ajarem et al., 2021). Photocatalytic activity of cobalt oxide NPs has been analyzed in the degradation of Remazol Brilliant Orange dye; these NPs (0.5 g) under 50 min irradiation time

degraded 78.45% of the dye (conc. 150 mgL⁻¹) (Medhi et al., 2020). *Coriandrum sativum* and *Allium sativum* belong to the family *alliaceae* which is famous for its culinary and medicinal properties. These two *sativum* species' seeds and cloves extracts are chosen to synthesize the cobalt oxide nanoparticles and the Photocatalytic activity of the synthesized nanoparticles was evaluated by degradation of Rhodamine Blue dye. The color of the dye Rhodamine Blue disappeared after UV irradiation for 60 min for Co₃O₄-A NPs at 544nm (Asha et al., 2021).

The average crystallite size of 39 nm of Co₃O₄ NPs was successfully synthesized using a facile and eco-friendly method utilizing leaf extracts from *C. medica* for the degradation of methyl orange dye using a very small amount of catalyst (0.006 g) using ultra violet radiation source. Approximately, 90% of the dye (Initial Conc. 10 mg L⁻¹) was degraded in 60 min at natural pH (6.5) (Siddique et al., 2021). Co₃O₄ NPs were successfully biosynthesized by a completely green process using aqueous leaf extracts of *Sageretia* as a chelating agent, and antibacterial antioxidant and enzyme inhibition applications were assessed (Haq et al., 2021). Antibacterial activity of green cobalt oxide nanoparticles by using *Ocimum sanctum* was estimated of zone inhibition by disc diffusion technique. The cobalt oxide nanoparticles possessed viable antibacterial activity against human pathogenic microbes, for example, *Pseudomonas aeruginosa* and *Escherichia coli*. The mechanistic antimicrobial actions of green synthesized Co₃O₄ NPs were measured by the disc diffusion approach (Ullah et al., 2019). Cobalt oxide nanoparticles are a typical transition metal oxide and important p-type semiconductors that have various applications including drug delivery, catalysis, magnetic resonance image, and heterogeneous catalysts (Medhi et al., 2020).

There are reports on cobalt oxide complexes being used as a catalyst material for different applications such as Nickel-cobalt oxide modified with reduced graphene oxide used for evaluating the performance and degradation for energy storage applications. It involves the transfer of electrons from the valence band to the conduction band on a semiconductor surface upon illumination with an appropriate wavelength of light (Abu-Zied & Alamry, 2019). The advantages of using Co₃O₄ NPs for wastewater treatments as colorimetric sensors for heavy metal ions in environmental systems in case of include simplicity, cost-effectiveness, and high sensitivity (Yahya et al., 2020). Nanoparticles have two key properties that make them attractive as sorbents such as due to their high surface area and unique super paramagnetism properties. Then, cobalt oxide nanoparticles are used as an adsorbent due to their small surface area, photocatalytic application, and paramagnetic property.

2.6. Water Pollution and Its Source

Water is contaminated by anionic constituents such as nitrate, phosphates, fluoride, chromate, sulfate, bicarbonate, and other toxic heavy metal ions that are a menace to both human health and environmental safety. Heavy metals in the environment are hazardous to human health and require better methods for detection and removal. Industrial effluent often contains a significant amount of toxic hexavalent chromium (Carolin et al., 2017). The introduction of heavy metal ions into water bodies constitutes a great risk to human health and the environment. The fresh and saline water is contaminated through waste streams of metallurgical operations, mining, agricultural activities, chemical industries, electroplating, and domestic wastewater (Buledi et al., 2021). The main toxic heavy metal contaminants are Pb^{2+} , Cd^{2+} , Cr^{6+} , Ni^{2+} , Zn^{2+} , Cu^{2+} , and Hg^{2+} ions (Saeed et al., 2020). Heavy metals are well-known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature. The mobility of these elements in the environment is to disturb their biogeochemical cycles and contaminates the aquatic and terrestrial ecosystems these metals are very reactive at low concentrations and can accumulate in the food web, causing severe public health concerns (Dukhinova et al., 2019). In general, there are two types of sources of heavy metals in the environment such as natural and anthropogenic sources. Natural sources such as soil erosion, volcanic eruptions, and the weathering of minerals are the major sources of heavy metals (Kgosiemang et al., 2020). The weathering process is influenced by the nature of the rock and the environmental conditions on which the concentration and composition of heavy metals largely depend, these rocks have high concentrations of Mn, Cr, Co, Cu, Ni, Zn, Sn, Cd, Hg, and Pb and simply release to the environments (Wang & Guo, 2020). Anthropogenic sources are the second and common source of heavy metal pollutants into territories and aquatic environments. It includes industrial emissions, mining, smelting, and agricultural activities like the application of pesticides and phosphate fertilizers and also, and the combustion of fossil fuels also contributes to the release of heavy metals such as cadmium (Cd) into the environment (Dukhinova et al., 2019). The application of chemical fertilizers and the combustion of fossil fuels also contribute to the anthropogenic input of heavy metals in the environment (Iravani & Varma, 2020). Figure 2 shows sources of heavy metal ions.

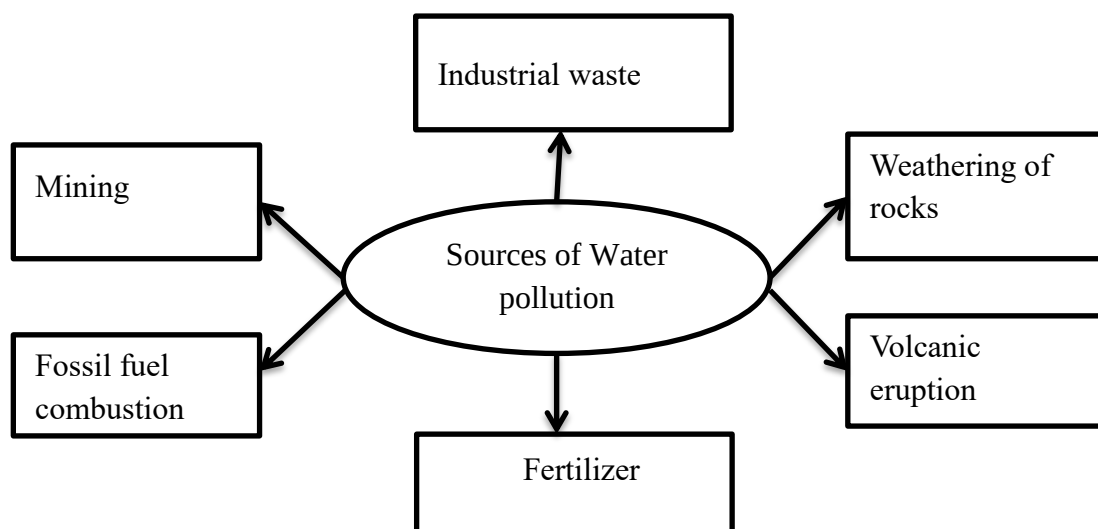


Figure 2: Main sources of heavy metal ions.

2.7. Toxic and Heavy Metal Ions

Heavy metals found in the environment and water bodies are lead ion (Pb(II)), cadmium ion (Cd(II)), and mercury ion (Hg(II)). These heavy metals commonly exist as divalent cations with an oxidation state of +2 in the aqueous solution as in Pb^{2+} , Cd^{2+} , and Hg^{2+} (Medhi et al., 2020). The toxicity and, methods of introduction of Pb(II) come from various sources, such as the smelting of ores, metal plating and battery industries, gasoline additives, and plumbing pipes (Kgosiemang et al., 2020). Exposure to lead can cause digestive problems, high blood pressure, kidney dysfunction, muscle, and joint pain, as well as memory and concentration problems in adults. Lead can damage the developing brains of infants and young children (Rahman & Singh, 2019). Cd(II) can be introduced to humans through the soil, water, food, and its long-term exposure can cause cancer and other organ system toxicity, such as cardiovascular, central and peripheral nervous, urinary, reproductive, skeletal, and respiratory systems (Review, 2017). The sources of cadmium include oil spills on boats, domestic disposals into water bodies, pesticides, and fertilizers, as well as industrial and domestic wastes. Hg(II) is intake in humans mainly from the consumption of estuarine and marine fish, as well as other seafood Methyl mercury exposure can cause neurological disorders, where exposed pregnant mothers can harm the development of the fetus, and in some cause severe cerebral (Kgosiemang et al., 2020). When the concentration of mercury in humans exceeds 0.1 mg/L, the effects on health include damaged nervous system, acrodynia, and protoplasm poisoning. In addition, according to (Zhang et al., 2020) even shorter exposure time to the high concentration of mercury can cause nausea, diarrhea, elevated blood pressure, and lung damage. Heavy metals

constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions and heavy metals are kept under the environmental pollutant category due to their toxic effects" on plants, animals, and human beings (Kgosiemang et al., 2020).

Chromium(Cr) is widely used in electroplating, leather tanning, metal finishing, and chromate preparation (Adegoke & Adekola, 2018). It is present in different forms most common forms are chromium (0), chromium (III), and chromium (VI). Chromium (III) occurs naturally in the environment and is an essential nutrient. Chromium (VI) and chromium (0) are generally produced by industrial processes and the recommended limit of chromium in potable water is 0.05 mg/l (Samuel et al., 2021). The prevalence of Cr(VI) in the environment greatly hangs on the pH as well concentration of the solution. The hexavalent chromium manifests in different anionic forms such as dichromate ($\text{Cr}_2\text{O}_7^{2-}$), hydrogen chromate (HCrO_4^-), and chromate (CrO_4^{2-}), which, are formed mainly due to the changes in the solution pH and concentration (Chakraborty et al., 2022). For example, chromic acid ($\text{H}_2\text{Cr}_2\text{O}_4$) is the major species if the pH is below 1 and if the pH is in the range between 2 and 6, dichromate ($\text{Cr}_2\text{O}_7^{2-}$) and hydrogen chromate (HCrO_4^-) is formed in an equilibrium state and the alkali condition (pH > 8) scenario only chromate ions remain. At a pH range of about 8-10, chromite occurs preferably as neutral species $\text{Cr}(\text{OH})_3$. $\text{Cr}(\text{OH})^-$ is prominent only at a particularly alkaline pH range (e.g., >12) (Samuel et al., 2021).

Chromium is a common pollutant introduced into natural waters due to the discharge of a variety of industrial wastewaters. Hexavalent chromium is mobile in the environment and highly toxic. Cr(VI) can easily penetrate the cell wall and exert its noxious influence on the cell itself (Begum et al., 2020). It's also, a source of various cancer diseases. At short-term exposure levels above the maximum contaminant level, Cr(VI) causes skin and stomach irritation or ulceration. Long-term exposure at levels above maximum contaminant can cause dermatitis, damage to the liver, kidney circulation, nerve tissue damage, and death in large doses (Samuel et al., 2021). The Cr (VI) toxicity generally originates from its oxidizing property as well as from its ability to form free radicals via the conversion/reduction process of Cr(VI) to Cr (III) within the cell. Inside the cells, Cr(VI) can potentially penetrate the cellular and nuclear membranes through the anion transporter, but normally the Cr(III) is inert due to the presence of the d3 electronic configuration (Zhang et al., 2020). The Cr(VI) leads to a greater impact on the DNA and thereby reacts very strongly than the Cr(III). The reduction of the Cr(VI) to Cr(III) occurs in the cytoplasm, nucleus, or blood and hence generates

free radicals that are intact with the DNA. In contact with the skin, Cr (VI) causes skin allergies, dermatitis, as well as dermal (Samuel et al., 2021).

2.8. Wastewater Treatment Methods

Waste streams contain toxic heavy metals such as chromium, cadmium, lead, mercury, nickel, and copper. Groundwater around many mining, plating and processing industries, nuclear fuel complexes, and military bases often gets contaminated with hazardous components. To meet environmental regulations, effluents or water contaminated with heavy metals must be treated before discharge (Crini & Lichtfouse, 2019). Several researchers develop many technological processes of remediation to bring these contaminant levels within the regulatory limit in the environment. Common conventional physicochemical methods such as coagulation, flocculation, precipitation, adsorption, ion exchange, electro-dialysis, and membrane separation can be applied in wastewater treatment schemes (Bashir et al., 2019). Chemical precipitation is effective and by far the most widely used process in the industry, because it is relatively simple and inexpensive to operate. In precipitation processes, chemicals react with heavy metal ions to form insoluble precipitates, the forming precipitates can be separated from the water by sedimentation or filtration, and the treated water is then decanted and appropriately discharged or reused.

The main disadvantage of hydroxide precipitation is the formation of large volumes of relatively low-density sludge, which presents dewatering and disposal problems (Crini et al., 2019). Reverse osmosis is a pressure-driven membrane process in which water passes through a membrane while the pollutant metal ions are retained. RO is more effective for metal ion removal from inorganic solutions and the method is effective at a wide pH range of 3-11 and a pressure range of 4.5-15. The major drawback of RO is the high power consumption due to the pumping pressures, and the restoration of the membranes (Wu, 2019). Ion exchange techniques are reversible interchange of ions between the solid and liquid phases that takes place. An insoluble substance (resin) removes metal ions from the solution and discharges other ions of likewise charge without changing any of the internal resin structures (Soliman & Moustafa, 2020a). Coagulation-flocculation involved a two-phase process to remove the stable colloids in water by forming larger aggregates that can be separated by the sedimentation process. The coagulation process is the first phase, which involved the addition of coagulants into the water to destabilize the particles by reducing the repulsive forces between the colloids. The second phase involved the flocculation process where the destabilized particles are bonded together to form flocs through the attraction of van der Waals force and to

remove turbidity of the water (Rhaman & Karim, 2020). However, most of the above-mentioned treatment technologies have limitations which include the generation of highly toxic sludge, high operation cost and maintenance expenditure, high energy requirements, long treatment times, and poor removal efficiencies. Among these methods, adsorption processes are effective and low-cost techniques for the removal of a variety of metal ions (Soliman & Moustafa, 2020a).

Table 2: Various conventional methods of wastewater treatment techniques

No of Technique	Advantage	Disadvantage	Reference
Chemical Precipitation	Simplicity of the process, safe operations, and low-cost	Generation of toxic sludge make extra operational cost for sludge disposal.	(Bashir et al., 2019)
Ion exchange	Recovery of metal values, selectivity, and less sludge produced.	Too expensive high cost and less number of metal ions removed	(Kgosieman g et al., 2020)
Electrocoagulation	No need for chemical additives. High selectivity, low pressure and Small space requirement.	Require high investments and an expensive electricity source for large scale	(Kgosieman g et al., 2020)
Membrane technology	Operated at low pressure condition with small space requirement. High separation selectivity	High operation cost due to membrane fouling	(Soliman & Moustafa, 2020a)
Electrochemistry	Efficient technology for the recovery/recycling of valuable metals , interesting method	High initial cost of the equipment. Cost of the maintenance, (sacrificial)	(Peng & Guo, 2020)
Ultrafiltration	High product throughput Environmentally friendly Easy scaling up	Membrane fouling	(Wu, 2019)
Adsorption with, new adsorbents	High metal binding capacities Low-cost, easy operating having wide pH range.	Low selectivity, production of waste products	(Zhang et al., 2020)

2.9. Adsorption

Adsorption is a mass transfer process that is a phenomenon of the sorption of gases or solutes by the solid or liquid surface absorbent. The adsorption on the solid surface is that the molecules or atoms on the solid surface have residual surface energy due to unbalanced forces (Sadegh et al., 2017). Adsorption is one of the most widely applied techniques for pollutant removal from contaminated media. The common adsorbents include activated carbon, molecular sieves, polymeric adsorbents,

and some other low-cost materials. When adsorption is concerned, thermodynamic and kinetic aspects should be involved to know more details about its performance and mechanisms (Hartmann et al., 2020). Adsorption is commonly employed as a polishing step to remove organic and inorganic contaminants in water and wastewater treatment. The efficiency of conventional adsorbents is usually limited by the surface area or active sites of the adsorbent, the lack of selectivity, and the adsorption kinetics. Nano-adsorbents offer significant improvement with their extremely high specific surface area and associated sorption sites, short intra particle diffusion distance, and tunable pore size and surface chemistry (Burakov et al., 2018). The surface functional groups of adsorbents (e.g., carboxyl, hydroxyl, and phenol) are the major adsorption sites for metal ions, mainly through electrostatic attraction and chemical bonding (Crini et al., 2019).

Chromium ions were removed from real wastewater (tannery and mine water) and synthetic matrices (synthetic and fortified solutions along with spiked groundwater and tap water) by synthesis of halloysite bentonite clay/magnesite nanocomposite using vibratory ball milling and calcination and the maximum adsorption capacity of activated cryptocrystalline magnesite was observed to be 199 mg g⁻¹ (Peng & Guo, 2020). *Thunbergia grandiflora* (Bengal clock vine) leaf extract has been used as green-resource to prepare Fe₃O₄ nanoparticles. SEM image showed agglomerated spherical (25 nm) particles and used to remove Acid blue 113 dye as an adsorbent. Batch adsorption of Acid blue 113 considering several factors conceded 95% removal within 3.5 hr of contact time at pH 2 with the adsorbent dosage of 1 g, the concentration of 25 mg/L, 150 rpm, and the temperature of 303 K (Hartmann et al., 2020). Co₃O₄ NPs with average crystallite size of 39 nm were successfully synthesized by utilizing leaf extracts from *Citrus medica* without impurities and used as photocatalytic activity for degradation of methyl orange dye. Approximately, 90% of the dye (Initial Conc. 10 mg L⁻¹) was degraded in 60 min at natural pH (6.5) by using of very small amount of catalyst (0.006 g) and ultra violet radiation source (Siddique et al., 2021).

2.9.1. Adsorption Isotherms

The isotherm models explained the relationship between the amount of a substance adsorbed per unit mass of adsorbent at constant temperature and its concentration in the equilibrium solution is called the adsorption isotherm. To study the relationship between the adsorbate and adsorbent concentration at equilibrium and the maximum adsorption capacity of adsorbent at a constant temperature and the equilibrium state is characterized by a concentration (loading) of adsorbate in the solid phase (q_e mg/g) which is in dynamic equilibrium with a solute concentration in the liquid

phase (C_e mg/L). The relationship between these q_e and C_e can normally be fitted to one or more equilibrium isotherm models (Alemu et al., 2018).

Langmuir and Freundlich's adsorption isotherm is the most commonly applicable model for the study of the adsorption process of pollutants up to now. Langmuir isotherm model assumes that adsorption cannot occur in any of the active sites, it only occurs at a fixed number of adsorption sites, and adsorption sites are equivalent to attracting adsorbate, and the Freundlich isotherm model assumes that adsorption can occur with the diverse surface, not fixed to the foundation of monolayer (Soliman & Moustafa, 2020b). The equilibrium state is characterized by a concentration (loading) of adsorbate in the solid phase (q_e mg/g) which is in dynamic equilibrium with a solute concentration in the liquid phase (C_e mg/L). A wide range of values of q_e versus C_e values may be obtained by varying the amount of adsorbent (m , g), the initial concentration of solute (C_0 , mg/L), and the volume of liquid. The relationship between these q_e and C_e can normally be fitted to one or more equilibrium isotherm models (Alemu et al., 2018).

Langmuir and Freundlich isotherm model

The Langmuir isotherm is the most widely used model and is based on the assumption that monolayer coverage occurs at specific homogeneous sites on the surface of the adsorbent having a constant number of identical and energetically equivalent adsorption sites without any departure into the plane of the adsorbent surface (Ajarem et al., 2021). While, Freundlich isotherm model describes heterogeneous surface adsorption. The energy distribution for adsorptive sites (in Freundlich isotherm) follows an exponential type function which is close to the real situation. The rate of adsorption/desorption varies with the strength of the energy at the adsorptive sites (Ajarem et al., 2021).

2.9.2. Adsorption Kinetic

The kinetic study has been used to evaluate the efficiency of adsorption in terms of the rate of adsorbate uptake onto an adsorbent. It provides information about the mechanism of adsorption, which is important for the efficiency of the process. In addition, the design of an adsorption system for water treatment may be influenced or even controlled by the adsorption kinetics (Ogata et al., 2020). The rate of adsorption may be controlled by mass transfer, intra-particle diffusion, or surface chemical kinetics. Several kinetic models for the liquid phase have been widely used to describe experimental data. These include pseudo-first-order, pseudo-second-order, and mass transfer/intra-

particle diffusion models (Peng & Guo, 2020). Adsorption kinetics for the hexavalent chromium ion were investigated in the pseudo-first-order, pseudo-second-order, and intraparticle diffusion models (Carolin et al., 2017)

2.10. Antibacterial Activity of Cobalt Oxide NPs

Cobalt oxide NPs synthesis from medicinal plants consists of bioactive compounds those phenolic compounds can result in the enhancement of the antibacterial potential of biogenic cobalt oxide relative to chemically produced counterparts (Samuel et al., 2020). Cytotoxicity of the cobalt oxide nanoparticles was confirmed by their dose-dependent response. The endocytosis mechanism for nanoparticles entry into the cells after entering the cells, the cobalt nanoparticles can leach Co^{++} ions that can lead to impairment in the nearby cellular organelle (Sundararaju et al., 2020). ROS generation is one of the primary causes of cellular disruption by oxidative stress and the active nanoparticle surface of cobalt oxide can readily generate ROS species by interacting with oxygen then hydrogen peroxide is formed under the bacterial membrane and double-strand DNA breaks (Ajarem et al., 2021). An antibacterial examination against both gram-negative and gram-positive bacteria has indeed been performed using the agar well diffusion method. These bacteria were cultivated on nutrient agar using a petridish to shape lawns. Then, 6 mm filter discs with 10 μg of $\text{Co}_3\text{O}_4\text{NPs}$, with 500 $\mu\text{g}/\text{ml}$ starting concentration were distributed on bacterial lawns, and Oxytetracycline (30 $\mu\text{g}/\text{ml}$) discs were often positioned in just the same dish as standard. Antibacterial activity was found to increase with increasing $\text{Co}_3\text{O}_4\text{NPs}$ concentration. *S. aureus* was less sensitive to MIC of $25.0 \pm 7.3 \mu\text{g}/\text{ml}$ *P. aeruginosa* is more sensitive to biogenic $\text{Co}_3\text{O}_4\text{NPs}$ with MIC values of $20.8 \pm 5.8 \mu\text{g}/\text{ml}$ (Ajarem et al., 2021).

2.11. Antibacterial Strains

The antibacterial potential of synthesized Co_3O_4 -NPs was analyzed initially by disk diffusion method using some Gram-positive and negative bacteria strains zone of inhibition was measured and compared with standard Ampicillins, which appeared as a clear area around the disks and strips (Hafeez et al., 2020). Gram-positive bacteria have highly variable growth and resistance patterns and they have a thicker peptidoglycan cell wall compared with gram-negative bacteria. It is a 20 to 80 nm thick polymer cell wall. The growth and attachment of bacteria on surfaces lead to contamination and/or infections such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Salmonella typhimurium*, and *Proteus Vulgarisy* (Sundararaju et al., 2020). *Staphylococcus aureus* is a gram-positive pathogenic bacterial strain and common bacteria that cause infections and diseases especially hurt skin and mucus membranes (Hafeez et al., 2020). Gram-negative bacteria have a thin peptidoglycan layer and have an outer lipid membrane or Lipopolysaccharide and gram-negative bacteria are more resistant against antibodies because their cell wall is impenetrable such as *Escherichia coli*, *Enterococcus faecalis*, and *Bacillus subtilis* (Pulingam et al., 2019). *E.coli*, enteric bacteria is one of the gram-negative bacteria, currently more implicated in many digestive tracts and urogenital infections with a high incidence of septicemia cases (Sundararaju et al., 2020).

3. MATERIALS AND METHODS

3.1. Chemicals and Reagents

The chemicals, reagents, and solvents used during this study include sodium hydroxide (NaOH, 98%) hydrochloric acid (HCl, 37%) ethanol (CH₃CH₂OH, 97%), cobalt nitrate hexahydrate (Co(NO₃)₂·6H₂O) precursor salt, nutrient agar, distilled water, ferric chloride (FeCl₃, 99%), and chloroform (99.9%) were used for samples preparation, samples cleanup and further characterization and adsorption studies. For batch adsorption experiments, stock solutions of Cr(VI) were prepared by dissolving analytical grade of potassium dichromate (K₂Cr₂O₇ 99.9%,), acetone, sulphuric acid (H₂SO₄, 98%), 1,5-diphenylcarbohydrazide (98%, Lobachemie, India). All the chemicals and reagents used were analytical grade.

3.2. Apparatus and Instruments

Apparatuses used for this work were Digital electronic balance (GF 6100, and company Tokyo Japan), Centrifuge, Oven, Shaker (SK300) Furnace (SX-2.5-12, China). The instruments used for this study were UV-Vis Diffraction reflectance spectroscopy (UV-Vis DRS, ElicoSL-150 spectrophotometer), X-ray Diffractometer (Model XRD-7000, Company Japan), Brunauer Emmett Teller (BET), and Scanning electron microscope-energy dispersive x-ray spectroscopy (FE-SEM, JEOL-JSM 6500F, made in Japan) Thermal gravimetric-Differential Thermal Analysis (TGA-DTA) (DTG-60H, Shimadzu Co., Japan) and Fourier transfer infrared spectroscopy (FTIR, Perkin Elmer 65 spectrophotometer).

3.3. Methods

3.3.1. Collection of *Ananas Comosus* Fruit Peel

Ananas comosus fruit peel was bought from the local market in Adama, Ethiopia. The fruit peel samples were collected and washed with distilled water several times to remove extraneous matter or contaminants. Then the washed peel was allowed to dry under shade at room temperature to remove residual moisture content and make it ready for grinding. After drying, the peel was crushed by an electrical grinding machine to produce a fine powder. The powder was packed within a plastic bottle followed by covering the whole body of the plastic bottle with aluminum foil.

3.3.2. Extract Preparation

Extraction of the peel was done by taking 30 g of peel powder followed by adding 450 mL of distilled water and placed the mixtures of the two components on a hot plate. The mixed components were heated at 60°C for 90 minutes until the required suspension was formed. Then, the extracted suspension was allowed to cool at room temperature followed by filtering it and placed the filtrates within a refrigerator at 4°C. Then the filtrate extract was used as a reducing and capping agent for the green synthesis of Co₃O₄ NPs (Dewi et al., 2019).

3.3.3. Preliminary Phytochemical Screening of *Ananas Comosus* Fruit Peel

The presence of phytochemicals like alkaloids, flavonoids, saponins, tannins, polyphenol or phenol, terpenoids, and reducing sugar in the aqueous extract of *Ananas comosus* fruit peel was evaluated using standard testing methods (Auwal et al., 2014) stated below.

Test for reducing sugar (Benedict test):-The presence of reducing sugar in peel extract was checked through the benedict test by using 2 mL of extract followed by benedict reagent addition drop by drop and heated for 2 min (Auwal et al., 2014).

Test for Phenol:- The presence of the phenolic functional group in peel extract was checked by using 5 mL of extract into a test tube and adding 7 mL of 3% of FeCl₃ drop by drop (Akpomie et al., 2021).

Test for Alkaloids (Wagner's test):- The presence of alkaloids in peel extract was checked through **Wagner's test** by using 10 mL of the extract was taken and stirred separately with 1% of 6 mL HCl on the water bath for 5 min and filtered into the test tube and finally, 4 drops of Wagner's reagent (Iodine in potassium iodide) was added (Anitha, 2019).

Test for Flavonoids (Alkaline Reagent Test):- The presence of flavonoid in the peel extract was checked through an alkaline reagent test by using 5 mL of extracts taken and treated with 5 drops of sodium hydroxide solution (NaOH) (Akpomie et al., 2021).

Test for Saponins:- 3 mL of peel extract was dissolved in 5 mL of distilled water and the mixture was shaken vigorously (Akpomie et al., 2021).

Test for Terpenoids:- The presence of terpenoids in the peel extract was checked through chloroform as a solvent by using 4 mL of extract dissolved in the 3 mL of chloroform, and 2 mL of concentrated sulfuric acid was added (Anitha, 2019).

Test for Tannins (Ferric chloride test):- Tannins were checked with a Ferric chloride test by using 3 mL of peel extract dissolved in 3 mL of distilled water, and 3 drops of 1% ferric chloride solution were added (Anitha, 2019).

3.3.4. Synthesis of Cobalt Oxide Nanoparticles

Cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and *Ananas comosus* fruit peel was used for the synthesis of Co_3O_4 NPs. To obtain ultra-fine nanoparticles of Co_3O_4 , optimizations were performed and the well-reduced Co_3O_4 nanosize was taken for further characterizations and applications. In synthesis, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was prepared and mixed within a reaction Erlenmeyer flask with peel extract approximately in 50:50, 33.33:66.66, and 66.66:33.3 means 1:1, 1:2 and 2:1 volume ratios. A small-sized magnetic bar was put inside the Erlenmeyer flask containing the extract and the precursor solution to enhance the reaction kinetics. The two components were stirred for 5 hrs at room temperature without applying any heat energy. After stirring time was completed, the pH of the formed suspensions was checked and controlled by adding appropriate quantities of NaOH solution to facilitate precipitation. Then, the resulting suspension was stirred again for 30 min to maintain the uniform distribution of the added base. The formed suspension was placed within a refrigerator for overnight to enhance the precipitate formation and then after the supernatant was decanted carefully. This was followed by centrifugation at 2000 rpm for 15 min and washed three times using ethanol and distilled water. Then, the precipitate was collected using a ceramic crucible dish followed by placing it into a drying oven for 3 hr at 100°C and then allowed to cool at room temperature. In the last, the dried precipitate was calcined for 5 hr using a muffle furnace at a temperature determined from TGA/DTA result. Finally, the nanoparticles were ground and ready for further characterizations and analysis. The general schematic representation of synthesis of Co_3O_4 was shown in Figure 3.

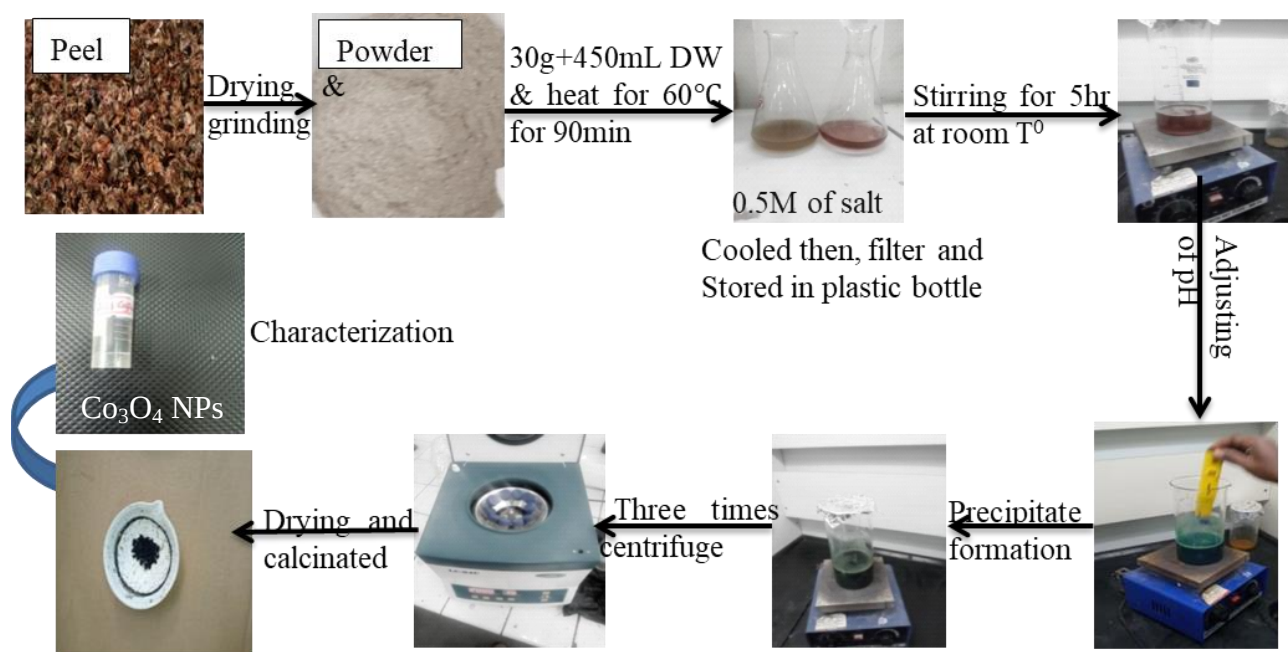


Figure 3: Diagrammatic representation of synthesized Co_3O_4 NPs.

3.4. Adsorption Study

3.4.1. Stock Solution Preparation

The stock solution of chromium (500 mg/L) sample was prepared by dissolving 0.68 g of analytical grade $\text{K}_2\text{Cr}_2\text{O}_7$ in 500 mL of distilled water. The standard solution of chromium with in various initial concentration (10, 20, 30, 40 and 50 mg/L) were prepared by appropriately diluting of 2, 4, 6, 8, and 10 mL of 500 mg/L stock solution with distilled water in 100 mL volumetric flask up to the mark respectively, and 0.25 gram of 1,5, diphenylcarbohydrazide was prepared in 50 mL volumetric flask with acetone as a standard solution. Then add 1 mL of 1,5, diphenylcarbohydride in each 40 mL Cr(VI) solution of known concentration (C_0) in 100 mL volumetric flask as complexation reagent and diluted with distilled water up to the calibration mark. Finally, a pink-purple-colored complex formed in an acidic medium, whose absorption was measured at 540 nm wavelength (Chakraborty et al., 2022).

3.4.2. Batch Adsorption Experiment

Batch adsorption experiments were carried out at room temperature. The effects of initial pH ranging from 2-12, contact time ranging from 30-150 min, biosorbent dosage ranging from 0.25-1.25 g, and initial Cr(VI) concentration ranging from 10 to 50 mg L^{-1} were studied. After each

biosorption experiment, samples were centrifuged at 300rpm for 60 min and filtrated using filter paper. The absorbance of the residual Cr (VI) concentration was determined by using UV-Visible spectrophotometer at 540 nm wavelength. Finally the adsorption efficiency and rate of Cr^{6+} ion on the surface of Co_3O_4 nano-adsorbent in an aqueous solution were determined (Asha et al., 2021).

3.4.3. Optimization Experiment

The parameter that influences the adsorption of hexavalent chromium ions such as pH of the solution, initial concentration of the chromium ions, contact time of sorbet, and adsorbent dose were chosen as an independent variable and the adsorption efficiency of hexavalent chromium ion is output responses (dependent variable) were determine by using 40 mL of chromium ion concentration from each standard solution at constant string speed of 300rpm. After that, the effects of each parameter were studied (Samuel et al., 2021)

3.4.4. Adsorption Isotherm

To study the adsorption isotherms the optimum conditions which were found from previous optimization experiments was applied only by varying initial concentration of Cr(VI) such as (10 up 50 mg/L) and the other parameter were constants (1g adsorbent dosage 120 min contact time, and at 2 pH). The data from the experiment were tested to fit into Langmuir or Freundlich isotherm models (Asha et al., 2021).

3.4.5. Adsorption Kinetics

The experiments were conducted for the optimum contact time from 30 to 150min and initial Cr (VI) concentration (10-50 mg/L). The other parameter was set constant such as pH 2, adsorbent dosage 1g, and agitation speed of 300rpm. The experimental data were analyzed either pseudo-first or pseudo-second-order kinetic models (Chakraborty et al., 2022).

3.5. Antibacterial Activity of Cobalt oxide Nanoparticles

The antibacterial activity of synthesized Co_3O_4 NPs was carried out on both Gram-positive and Gram-negative bacteria strains. The Gram-positive bacteria *Staphylococcus aureus*, and *Streptococcus pyogen*, and the Gram-negative bacteria strains *Escherichia-Coli*, and *Pseudomonas aeruginosa*, bacteria were used for antibacterial study. They were obtained from applied biological department laboratory, at Adama Science and Technology University. The bacteria were incubated using nutrient agar and carried out using disc diffusion methods.

3.5.1. Media Preparation and Bacterial Culture

Nutrient agar media were prepared by dissolving of 1.5 g nutrient agar within 100 mL distilled water and then sterilized using autoclave at 120°C for 20 min. After cooling, about 20 mL of medium are added to sterilized Petri dishes. Bacterial cultures were maintained on nutrient agar at 37°C. Four bacterial strains were mixing in nutrient broth and shaken gently at 160rpm to allow equal mixing of bacteria cells with agar and growth for 24hr at 37°C (Hafeez et al., 2020).

3.5.2. Disc Diffusion Method

Antibacterial activity of biosynthesized Co_3O_4 NPs in three volume ratios (1:1, 1:2, and 2:1) on Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogen*) and Gram-negative (*Escherichia coli* and *P. aeruginosa*) bacteria were tested by the disc diffusion method based on the procedures reported (Abbasi et al., 2021). Bacterial suspension was prepared until the concentrations of bacterial suspensions were achieved to 1×10^8 colony forming units (CFU) mL^{-1} by comparison with 0.5 McFarland standards. Different concentration such as 100, 75 and 50 μg of each prepared samples were dissolved in 1 mL dimethyl sulfoxide (DMSO) in a test tube as the negative control (NC). The standardized culture of test bacteria was first evenly spread onto the surface of the nutrient agar plates using sterile cotton swabs. 6 mm diameter of filter paper was prepared and placed in different areas of the disc and 20 μL , of Co_3O_4 nanodrug was saturated on 6 mm paper from each concentration. Ampicillin was placed on even lawns as a positive control. The plates were then turned upside down and incubated at 37°C for 24 hr in an incubator. Finally, antibacterial activity was then evaluated by measuring the diameter (mm) of the inhibition zone (IZ) around the disc against positive control and each bacteria strain were measured using a ruler and compared results (Bekele et al., 2022).

4. RESULTS AND DISCUSSIONS

4.1. Phytochemical Analysis of the Peel Extract

The qualitative analysis of the bioactive compound present in the *Ananas comosus* peel extract was done. The bioactive compounds such as Reducing sugar, Alkaloid, phenol (polyphenol), Tannins, Flavonoids, Saponins, and Terpenoids in the peel extract were confirmed, and they showed color changes with their respective tests. Based on the result the natural existence of phytochemicals in the peel extract was used as reducing and capping agents during the synthesis of Co_3O_4 NPs. The results of qualitative phytochemical analysis of *Ananas comosus* peel extract were shown in Figure 4, and Table 2: Which revealed the presence of secondary metabolites that could play a great role during the synthesis of Co_3O_4 NPs as a template (Akpomie et al., 2021).

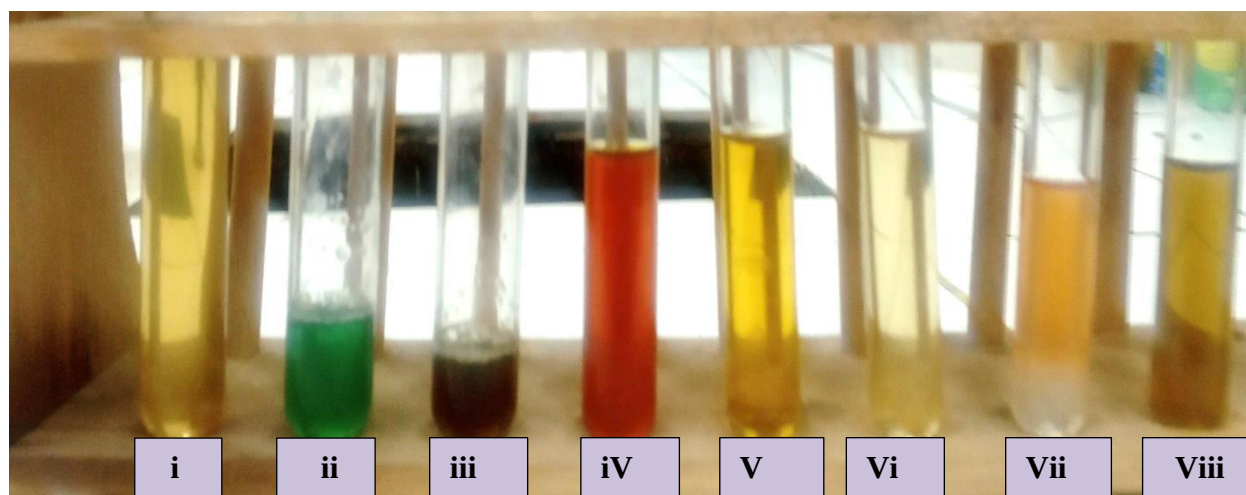


Figure 4: The color change observed when the peel extract of *Ananas comosus* was tested for the presence (ii) Reducing sugar, (iii) Phenol (iv), Alkaloids, (v), Flavonoids, (vi) Saponins (vii) Terpenoids (viii) Tannins.

Table 3: Phytochemical screening of ananas comosus peel extract

N _O	Phytochemical constituent	Reagent added	Color observed	Results
1	Reducing sugar	Benedict test	Blue-green	+
2	Alkaloid	Wagner reagent	Reddish	+
3	Polyphenol	Ferric chloride	Dark green/ black	+
4	Flavonoids	Alkaline Reagent Test	Yellow	+
5	Tannins	Ferric chloride	Green	-
6	Saponins	Distilled water	Stable persistent	-
7	Terpenoids	Chloroform and H ₂ SO ₄	Reddish brown	+

+' indicates the presence and '-' indicates the absence

4.2. Characterization of Cobalt Oxide Nanoparticles

4.2.1. Thermal Gravimetric-Differential Thermal Analysis (TGA-DTA).

The thermal stability of the synthesized Co₃O₄ NPs is a crucial factor to estimate their ability to sustain through various applications (Chekin et al., 2016). Therefore, the Thermogravimetric analysis was conducted from 50-800°C using 12 mg uncalcinated Co₃O₄ (1:1) NPs. Figure 5 shows, the TGA-DTA analysis of the uncalcined Co₃O₄ NPs (1:1). TGA curve shows mass loss of the sample whereas the DTA curve indicates the energy gain or loss during the process from Figure 5, the TGA curve of Co₃O₄ NPs was experienced weight loss in three main steps. The first curve indicates the temperature starting from 100°C up to 275°C was an essential loss of water molecules adsorbed on the surface and at which, only about 3.48 mg mass of the decomposition was observed. The second step showed the compactions and volatilization of organic molecules and the formation of Co₂O₃ NPs and the maximum mass loss was 6.18 mg at 275°C to 430°C. In the third step, Co²⁺ changed to Co³⁺ and about 1.2 mg of mass loss at a temperature of 430°C to 600°C was detected which indicates the formation of nano crystalline Co₃O₄ as the decomposition product. However, Co₃O₄ NPs was calcinated at 500°C based on thermal gravimetric analysis data in this work. DTA of Co₃O₄ NPs showed maximum exothermic peaks at 42.41 μV, and the fastest mass loss of 6.618 mg/min occurred at 334.28°C and the reaction terminated in the range of 600-800°C and this result is in agreement with the previous report (Sundararaju et al., 2020)

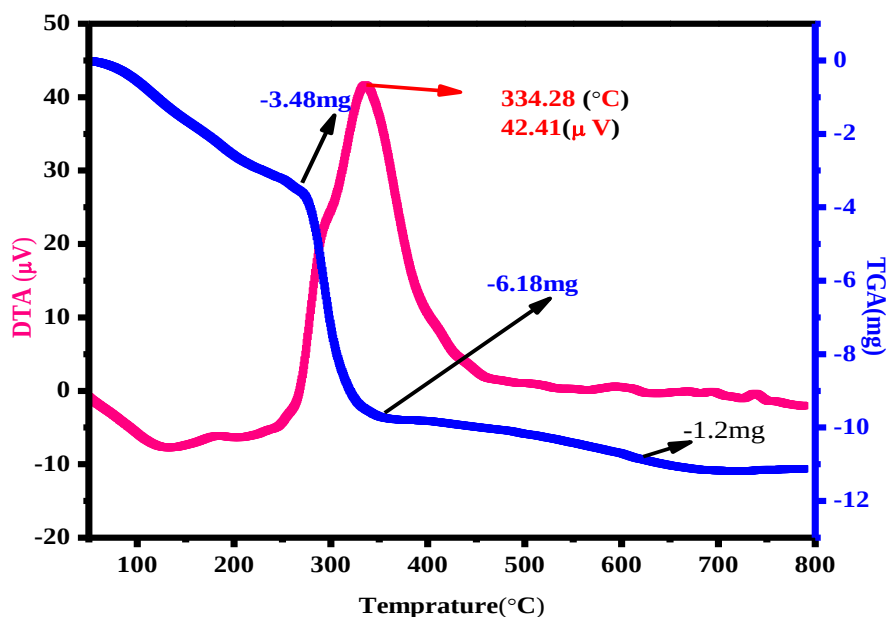


Figure 5: TGA-DTA graphs of Uncalcinated Co₃O₄ (1:1) NPs.

4.2.2. X-Ray diffractometer (XRD) Analysis of Calcinated Co₃O₄ NPs

Figure 6: shows the XRD pattern of Co₃O₄ NPs using three volume ratios. The XRD of Co₃O₄ (1:1) NPs showed peaks appeared at 2θ values of $\approx 18.97^\circ$, 31.42° , 36.81° , 38.98° , 44.61° , 59.60° , 65.30° , and 76.10° . The XRD of Co₃O₄ (1:2) NPs showed peaks appeared at 2θ values of ≈ 18.91 , 31.48 , 36.86 , 38.7 , 44.92 , 55.52 , 59.2 , and 77.7° . Also the XRD of Co₃O₄ (2:1) NPs showed peaks appeared at 2θ values of ≈ 19.06 , 31.49 , 37.01 , 42.37 , 55.95 , 59.33 , 65.42 and 77.43° corresponding to the Miller indices value of (111), (220), (311), (222), (400), (422), (511), and (440), respectively. The diffraction peaks of Co₃O₄ NPs were found to be in good agreement with (JCPDS card No. 042-1467), which confirms the purity of the green synthesized Co₃O₄ NPs without the formation of any secondary phases and the result found to be in good agreement with the previously reported work (Bekele et al., 2022). According to Scherer's equation, the calculated average crystallite size of synthesized Co₃O₄ NPs was found to be 12.58, 13.40, and 22.05 nm for the volume ratio of 1:1, 1:2, and 2:1, respectively, and the result is presented in Table 4.

$$D = \frac{K\lambda}{B \cos \theta} \dots\dots\dots 1$$

Where D is the average crystalline diameter K'' is the Scherer's constant (0.94), λ (0.15406 nm) is the X-ray wavelength, β is the full-width half maximum (FWHM) of the peak in radians, and θ is the Bragg's angle in degree. Among the three-volume ratio of Co₃O₄ NPs, (1:1) ratio has a better

and smaller crystalline size than other counterparts. This might be contributed due to the presence of an equal volume of capping /reducing agent within salt precursor and more homogenization between the two components. Furthermore, the XRD spectra of Co_3O_4 (1:1) NPs possessed peak was sharp and high heights compared to the other counterparts and this indicates the material has more crystalline size nature. On the other hand, the XRD spectra of Co_3O_4 (2:1) NPs showed broad and short peak which indicate that the synthesized nanoparticles are less crystalline (Hafeez et al., 2020) .The average crystal size of the biosynthesized Co_3O_4 NPs has decreased when the ratio of the peel extract increased because the effective capping and stabilizing agent has able to hinder the aggregation. In this study, the volume of precursor salt increases to confirm the size enhancements of the NPs. The absence of additional peaks on the synthesized NPs indicated the degree of purity of the synthesized Co_3O_4 NPs (Bekele et al., 2022; Siddique et al., 2021).

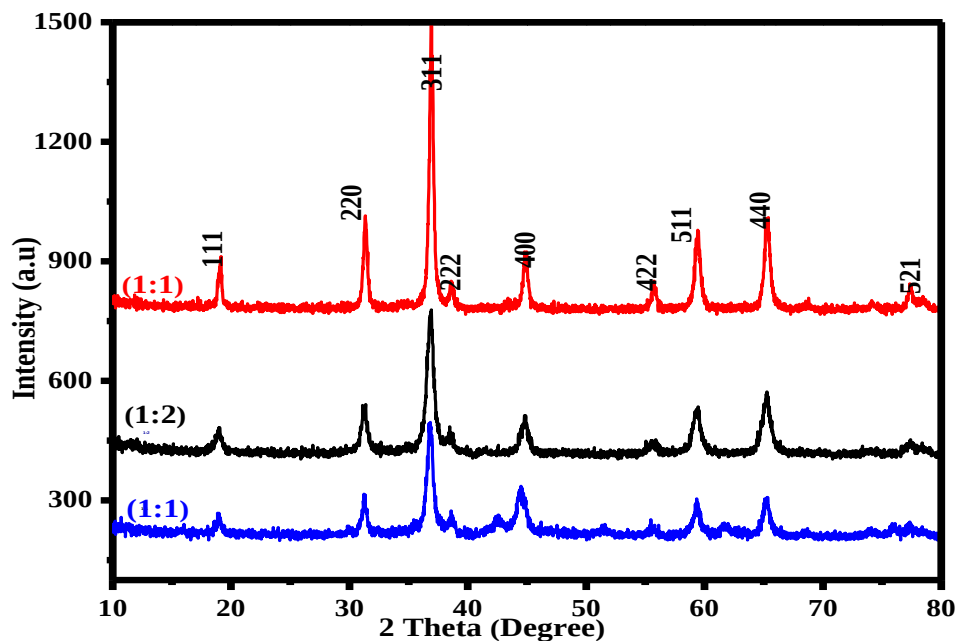


Figure 6: XRD pattern of *Ananas comosus* peel extract synthesized Co_3O_4 NPs.

Table 4: Calculated crystal size of synthesized Co_3O_4 NPs in three volume ratios.

Ratios of Co_3O_4 NPs	2θ (degree)	FWHM (θ)	FWHM (3.14/180)	hkl	D(nm)	Average size(nm)
1:1	31.42	0.6879	0.012006	220	12.7131	12.58
	36.81	0.7600	0.013265	311	12.9587	
	59.60	0.7900	0.013788	511	12.0854	
1:2	31.48	0.6057	0.010571	220	14.4376	13.40
	38.70	0.8800	0.011053	311	10.1885	
	44.92	0.6333	0.015359	400	15.5507	
2:1	31.49	0.3880	0.006772	220	22.5455	22.05
	42.37	0.4629	0.006735	400	21.2853	
	37.01	0.3859	0.008079	311	22.3324	

4.2.3 Scanning Electron Microscopic (SEM) Analysis

Scanning electron microscope results confirmed the surface morphologies of biosynthesized Co_3O_4 NPs and the results are presented in Figure 7(a-c). Figure 7(a) shows the SEM image as-synthesized Co_3O_4 NPs using 1:1 ratio by volume of the precursor and peel extract. The image shows spherical shapes and is more deeply homogenized than the other counterpart of Co_3O_4 NPs. In addition to this, the morphology of Co_3O_4 NPs synthesized from 1:2 volume ratios of reactants in Figure 7(b) showed predominantly spherical shape which might be due to the presence of more peel extract that stabilizes the formation of NPs and prevents their aggregation. The agglomeration of the particles is dependent upon the nature of compounds present in the extracts and attraction between the biomolecules and metal salts resulted in the aggregation of the NPs (Siddique et al., 2021). In the latter, the SEM image of Co_3O_4 NPs synthesized from 2:1 reactant ratio by volume in Figure 7(c), showed a honeycomb-like structure and more crystalline than the other ratio of Co_3O_4 NPs as the amount of the salt precursor was much greater than the amount of peel extract contains it and form aggregation. In the previous report, this aggregation or agglomeration may be caused due to polarity and electrostatic attraction between biomolecules and Co_3O_4 NPs (Siddique et al., 2021).

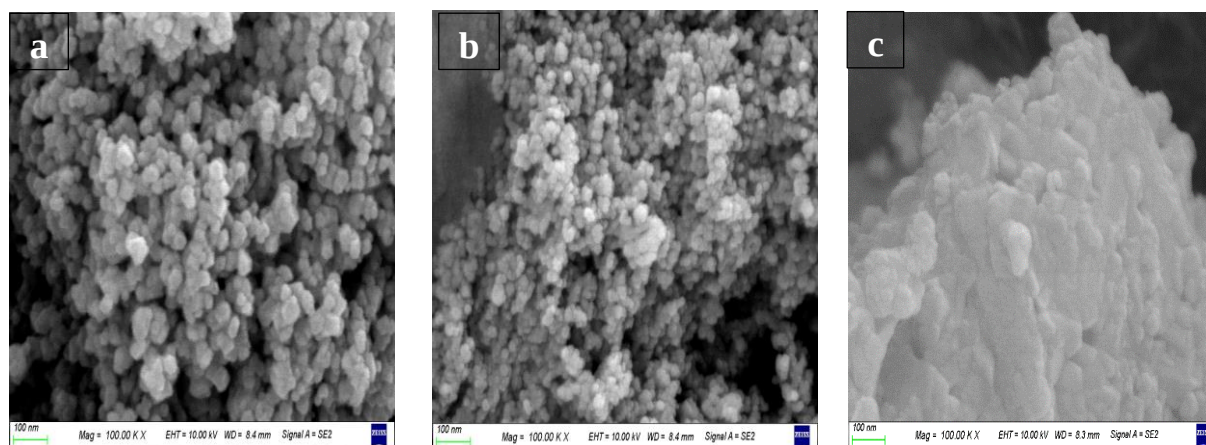


Figure 7: The SEM image of Co_3O_4 NPs a (1:1), b (1:2) and c (2:1) volume ratios.

4.2.4. Energy Dispersive X-Ray Spectroscopy (EDS) Analysis

The energy dispersive X-ray analysis of Co_3O_4 (1:1) NPs was shown in Figure 8(a-c) and the sample obtained from SEM analysis in Figure 8(a) with an equal volume ratio of salt precursor and peel extract. The EDS analysis of Co_3O_4 NPs was carried out using an internal standard at the energy of 0 up to 10 KeV. The EDS studies of results in Figure 8(b) confirm that Co_3O_4 NPs contain Co and O as major elements. Moreover, the EDS analysis of Co_3O_4 NPs in Figure(c) showed two major peaks and other small peaks on the graph. The first strong peaks at 0.4 and 0.8 keV confirmed the presence of O and Co dominantly. The results indicate that the reaction product was composed of high purity Co_3O_4 NPs, and the composition obtained from EDS analysis of the normalized spectrum was Co (74.51%) and O (25.49 %) proving that the produced nanoparticle was the highest purified form. The EDS analyses in this study showed similar results to the previous report (Asha et al., 2021). The EDS also confirm additional minor peaks in trace amounts in Figure 8(c) that might be due to the presence of other impurities like K and Zn, and Fe as contaminants from equipment and preparation of standards.

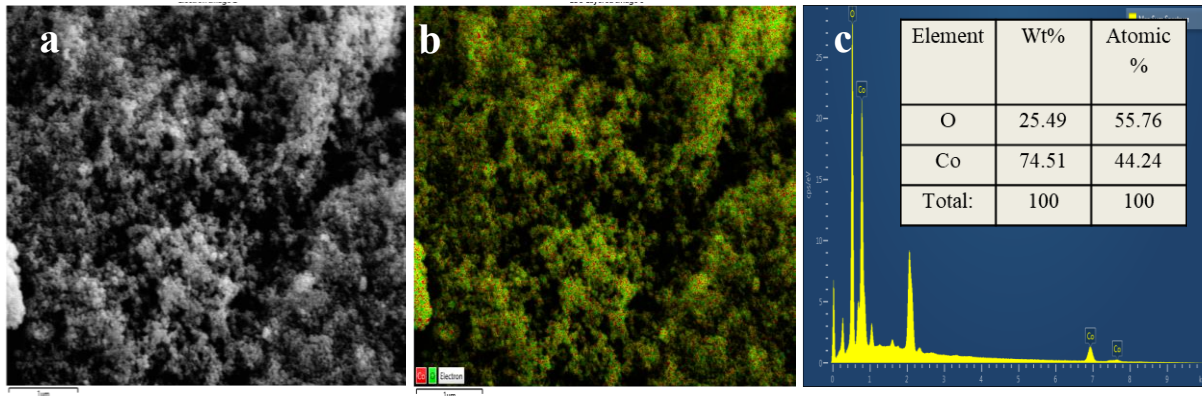


Figure 8: EDS image of Co₃O₄ (1:1) NPs, (a) SEM image of target sample, (b) image of Co₃O₄ NPs composition and (c) elemental composition.

4.2.4. Ultraviolet-visible Diffuse Reflectance Spectroscopy (UV-Vis DRS) Analysis.

The energy band gap of synthesized Co₃O₄ NPs was estimated by using ultraviolet-visible diffuse reflectance spectroscopy as shown in Figures 9(a -b). Figure 9(a), shows the reflectance spectra of synthesized Co₃O₄ NPs in different volume ratios characterized in the range of 200-800 nm. The peaks are assigned to the band gap of Co₃O₄ NPs owed to electron transitions from a lower energy state to a higher energy state. Here the Co ion is occupied in the distinct oxidation energy levels (Anuradha & Raji, 2021), so these ions perform as a P-type semiconductor, and its energy band gaps were evaluated from Tauc formula and extrapolation procedure.

$$(\alpha h\nu)^{\frac{1}{n}} = k (h\nu - E_g) \dots\dots\dots 2$$

Where α is the absorbance coefficient which similar to kubelka -munk function $F(R)$ is the ratio of absorption coefficient Kc and scattering coefficient (S) in m^{-1} R is the % of reflectance obtained from DRS data, h is the Planck constant, k is the absorption constant for a direct transition, $h\nu$ is the absorption energy, and E_g is the band gap energy. The band gap energy of the different volume ratios of Co₃O₄ NPs was estimated by ascertaining the intercept line ($(\alpha h\nu)^{1/2} = 0$) on the energy ($h\nu$) axis. The plot of $(\alpha h\nu)^{1/2}$ vs ($h\nu$) is shown in Figure 9(b). During the calcination of synthesized Co₃O₄ NPs the temperature increase, change the phase transition of Co³⁺ ions from low-to-high spin sites, and produces a bounded transition to arbitrarily distributed Co³⁺ and Co²⁺ on tetrahedral phase and octahedral phase of the structure. The energy band gaps of the studies were deduced to be 3.33, 2.95, and 2.70 eV for the volume ratios 1:1, 1:2, and 2:1 respectively. It proves the purity and

optical properties of synthesized Co_3O_4 semiconductors and is used for various electronic applications. In addition to this, the 1:1 volume ratio has larger band gap energy and it has high quantum confinement as compared to its counterparts since small-size nanoparticles had a high surface area to volume ratio and that helps to reflect the incident light. The band gap energy analysis confirmed the formation of stable Co_3O_4 NPs (Bekele et al., 2022). If the size of the particle is very small light interacts with the samples instead of absorbing with parts of the light scattered and reflected.

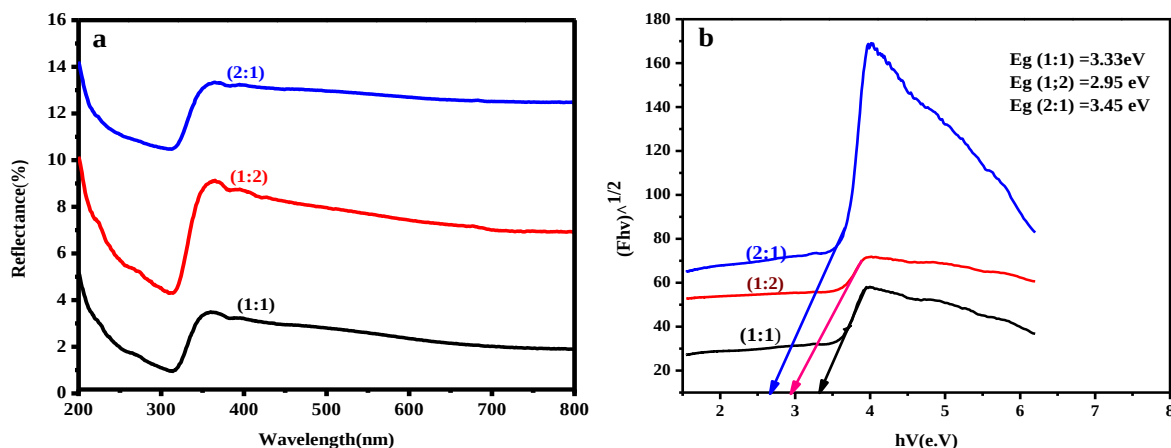


Figure 9(a): UV-visible reflectance spectra and (b), energy band gap of Co_3O_4 NPs.

4.2.5. Fourier Transform Infrared (FTIR) Analysis.

Figure 10(a-c), shows the functional group of calcinated and, uncalcinated synthesized Co_3O_4 (1:1) NPs and the extracts of *Ananas comosus* peel powder which were recorded in the range of $4000\text{--}400\text{ cm}^{-1}$. The FT-IR spectra of *Ananas comosus* peel extract used for the synthesis of Co_3O_4 NPs were indicated in Figure 10(a). The broad peak observed in peel extract of *Ananas comosus* around 3395 cm^{-1} spectrum represents the O-H stretching confirming aliphatic carboxylic acid. The prominent levels of absorption peak located at around 2921 cm^{-1} reveal the presence of sp^3 C-H stretching vibrations, of an aromatic aldehyde. The peak observed around $2360\text{--}2108\text{ cm}^{-1}$ represents the amide ($\text{C}\equiv\text{N}$) functional groups. The C=O stretching of the carbonyl functional group could be observed at 1717 cm^{-1} and the C=C functional group for an extract of ananas peel powder at 1606 cm^{-1} . The C-O stretched functional group of the extract was located at 1242 cm^{-1} and the C-C functional group could be observed around 1029 cm^{-1} . Figure 10(b) shows FTIR spectra of uncalcinated Co_3O_4 NPs that exhibit the absorption peaks at 3365 cm^{-1} may be due to O-H stretching. The band at 1587 cm^{-1} due to C=C stretching and the bands at 1354 cm^{-1} assigned to C-H Stretching. The peaks located at 1041 cm^{-1} due to the presence of the C-C functional group. The

band at 827 cm^{-1} corresponds to the O-Co-O bridging vibration and at 560 cm^{-1} the stretch of Co-O vibration (Asha et al., 2021). Figure 10(c) also, shown calcinated Co_3O_4 NPs at 500°C and have shown some peak on the graph at 1380 nm and 870 nm that might indicate the figure print region that shows metal and peel extract interaction and Co-O reaction during NPs formation. The vibration stretching band for the high annealed temperature was noticed around 650 and 547 cm^{-1} , which are assigned to the elongation of the Co^{3+} -O bond and Co^{2+} -O stretching, respectively.

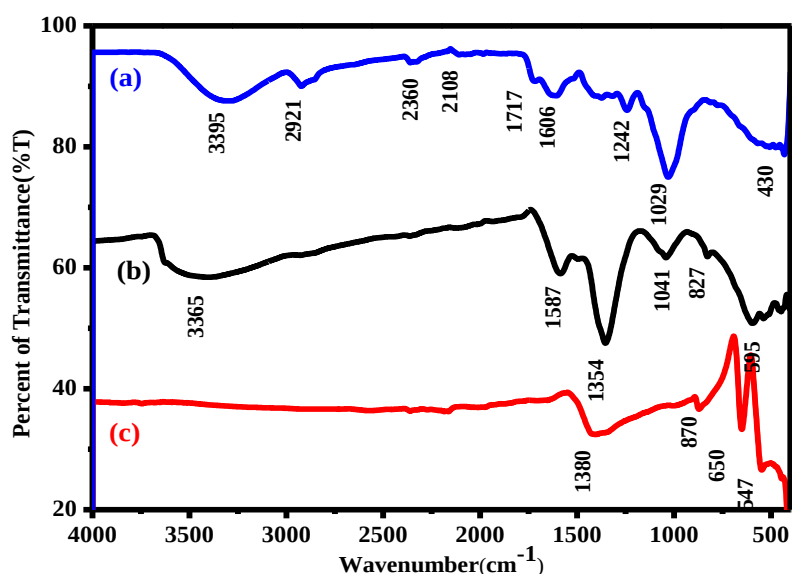


Figure 10: FTIR spectra of (a) *Ananas comosus* peel powder extract (b) uncalcinated Co_3O_4 NPs and (c) calcinated Co_3O_4 NPs

4.2.6. Brunauer-Emmett-Teller (BET) Analysis

The porosity and specific surface area of Co_3O_4 NPs were analyzed using Brunauer-Emmett-Teller (BET) and the result is shown in Figure 11, and Table 5. The specific surface area of Co_3O_4 NPs in the 1:1, 1:2, and 2:1 volume ratios was found to be 24.71 , 23.17 , and $22.50\text{ m}^2/\text{g}$ with a pore volume of 0.149 , 0.152 , and, $0.122\text{ cm}^3/\text{g}$, respectively. Furthermore, the pore size distribution was confirmed to be 19 , 18 , and 16 nm in diameter. The specific surface area is increase as the particle size becomes small. However, the specific surface area is also, increase if the particle has pore density (Lemecho et al., 2022). BET method is based on the amount of gas needed to form the saturation monolayer on the powder surface and surface area is related to the mass of adsorbed at a monolayer capacity and Barrett-Joyner Halenda (BJH) method was used for the determination of the average pore size distribution and volume analysis (Chakraborty et al., 2022). The surface area

is determined via the adsorption of nitrogen at the accessible particle surfaces and the total surface area and specific surface area of the adsorbent during the adsorption-desorption process were calculated using the following BET isothermal equations.

$$\text{Surface area or } S_{\text{total}} = \frac{V_m N_s}{V} \text{ and BET or specific Surface area} = \frac{S_{\text{total}}}{a} \dots\dots\dots 3$$

Where, V_m monolayer adsorption capacity N_s Avogadro number S cross-section area of adsorbing species on adsorption (0.162 nm^2 for N_2 , V molar volume (22.414 L) and “ a ” is mass of adsorbent.

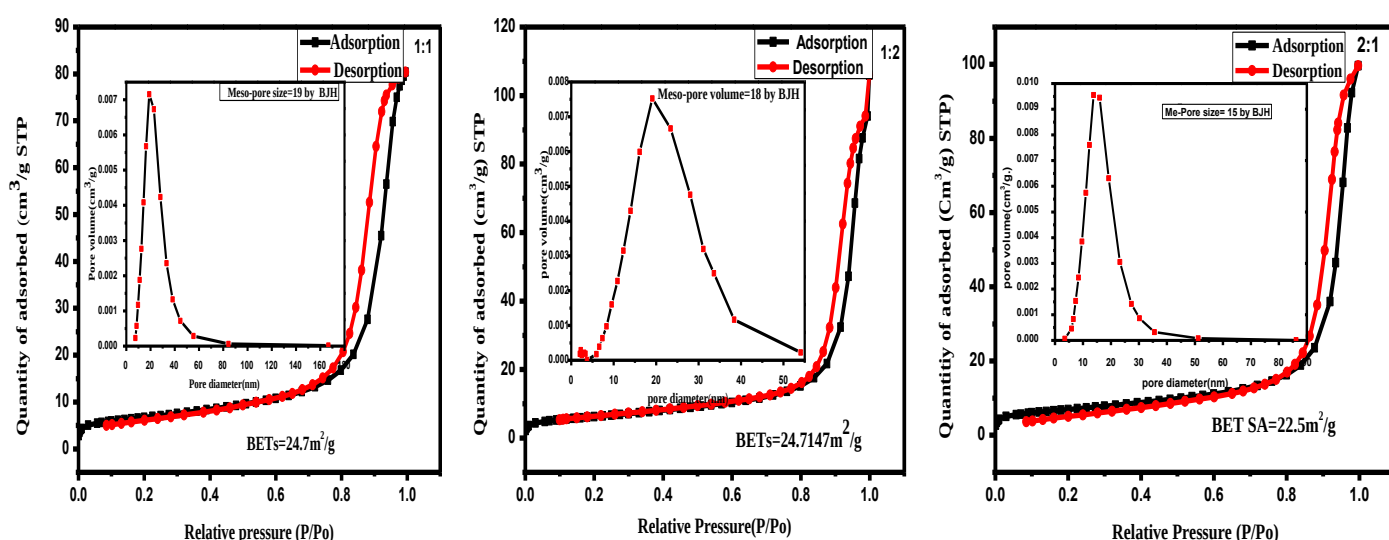


Figure 11: BET surface area and BJH pore size analysis of Co_3O_4 NPs.

From the N_2 adsorption-desorption results, the BET surface area of Cobalt oxide nanoparticles in different volume ratios such as 1:1, 1:2, and 2:1 were 24.7, 23.7, and $22.5 \text{ m}^2/\text{g}$ respectively. Therefore, the results of BET analysis of Co_3O_4 NPs in 1:1 volume ratio had a higher specific surface area to volume ratio than other synthesized cobalt oxide NPs due to formation of small crystal size (Kara et al., 2020).

Table 5: Surface area and pore size of synthesized Co_3O_4 NPs by (BET) (BJH) analysis,

The volume ratios of Co_3O_4 NPs	Mass of adsorbent	Specific surface area	Average pore volume	Mean pore diameter
1:1	0.0881 g	24.7147 m^2/g	0.149453 cm^3/g	19 nm
1:2	0.1422 g	23.7725 m^2/g	0.152923 cm^3/g	18 nm
2:1	0.1560 g	22.5033 m^2/g	0.122681 cm^3/g	16 nm

4.3. Adsorption Experiment

Determination of the residual chromium concentration was done using UV-Vis spectrophotometer at the wavelength of 540 nm and assisted with 1,5- diphenylcarbohydrazide. Hexavalent chromium reacts with 1,5- diphenylcarbohydrazide to form a reddish purple color in an acidic solution and quantified its absorbance by using a UV-Vis spectrophotometer. The calibration curve was done in various concentrations (5, 10, 20, 30, and, 40 mg/L) of chromium solution in mg/L show in Figure 12. The experiments were repeated three times and the average data were plotted and fitted with high regression coefficient value ($R^2 = 0.998$)

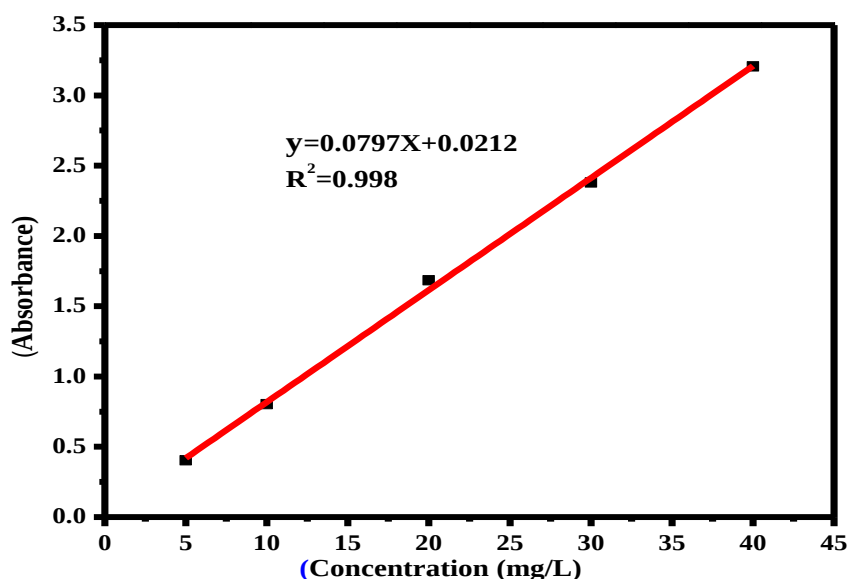


Figure 12: Calibration curves of prepared standard solution.

The removal efficiency and adsorption capacity of each synthesized Co_3O_4 nano-adsorbents such as 1:1, 1:2, and 2:1 by volume ratios were evaluated based on the calibration curve in Figure 12. Therefore, the removal efficiency were 74.8, 73.2, and 68% using 1:1, 1:2, and 2:1 volume ratio of synthesized Co_3O_4 nano adsorbent from cobalt nitrate hexahydrate and *Ananas comosus* peel extract respectively. The parameter that influences the adsorption process was kept constant such as 0.5 g

of adsorbent dose each different volume ratio of Co_3O_4 nano-adsorbent, 40 mL of 20 mg/L concentration of Cr (VI) at pH=4 value and 45min of contact time for 150 rpm agitation speed. After performing the experiment maximum removal efficiency and adsorption capacity of Cr (VI) were recorded using (1:1) ratio of Co_3O_4 nano adsorbent because of the small crystalline size and high surface area to volume ratio than the other counterpart of cobalt oxide nano adsorbent as shown in Table 6 and supported by Figure 13.

The percentage removal and adsorption capacity or metal uptake Q_e (mg/g) at equilibrium times were determined by using equations 4 and 5 respectively.

$$\text{The \% removal efficiency of Cr (VI)} = \frac{C_o - C_e}{C_o} \times 100 \dots\dots\dots 4$$

$$\text{Adsorption capacity (} Q_e \text{)} = \left(\frac{C_o - C_e}{M} \right) \times V \dots\dots\dots 5$$

Where, C_o and C_e are the concentrations of Cr(VI) at the initial and after the adsorption process V is the volume of chromium and M mass of adsorbent in gram that is taken.

Table 6: The removal efficiency and adsorption capacity of Co_3O_4 NPs

Volume ratios of Co_3O_4 NPs	Adsorbent dose(g/mL)	C_o (mg/L)	Absorbance	C_e	Maximum removal efficiency (%)	Adsorption capacity (%)
1:1	0.5	20	0.422	5.0288	74.8	30
1:2	0.5	20	0.448	5.3550	73.2	29.3
2:1	0.5	20	0.526	6.3337	68	27

The percentage removal efficiency of synthesized cobalt oxide nano-adsorbent in different volume ratios is described below in Figure 13 in bar graph form.

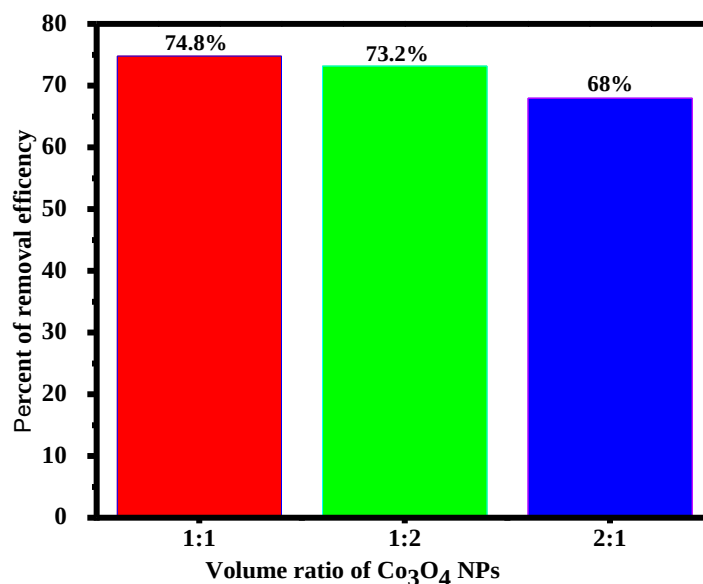


Figure 13: The removal efficiency of different volume ratios of Co₃O₄ NPs.

4.4. Optimization of Parameter

4.4.1. Effect of pH

In aqueous solutions, Cr ions exist in different oxidation states and it commonly occurs in one of three ionic forms, i.e., chromate (CrO_4^{2-}), hydrogen chromate (HCrO_4^-), and dichromate ($\text{Cr}_2\text{O}_7^{2-}$). Figure 14 shows the removal efficiency of Co₃O₄ nano-adsorbent in (1:1) volume ratio at different pH values. As the pH value was increased from 2 to 12, the removal efficiency decreased from 88.9% to 76% due to the changes in surface charges of the adsorbent and chromium speciation as a function of pH. At pH 2 the adsorption efficiency of Co₃O₄ (1:1) nano-adsorbent was 89.9% at low pH and the removal efficiency also decreased with an increase in pH. The other parameters were kept constant as adsorbent dosage was 0.5 g initial concentration of Cr ions 20 mg/L and contact time at 60 min at 300 rpm. At low pH values, the dominant species of Cr(VI) in solution is HCrO_4^- . This is converted to CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ ions as the pH increases (Kara et al., 2020). The adsorption free energy change for HCrO_4^- is lower than those of CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ ions. Therefore, HCrO_4^- is more easily adsorbed than CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ at lower pH values. In addition to this at low pH, the concentration of H^+ increases on the surface of Co₃O₄ nano adsorbent and becomes more cationic adsorbent. Then the anionic adsorbate Cr (VI) can simply interact with the surface of cationic nano adsorbent Co₃O₄, due to the electrostatic attraction of positively charged surface functional groups of adsorbent with HCrO_4^- species. At a high pH value, the concentration of OH^- ion increases on the surface of Co₃O₄ nano-adsorbent and the concentration of HCrO_4^- is shifted to other forms such as

CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$. Then the adsorption free energy change of the cationic surface becomes decrease and repulses the anionic adsorbate of Cr (VI) and the removal efficiency becomes decrease.

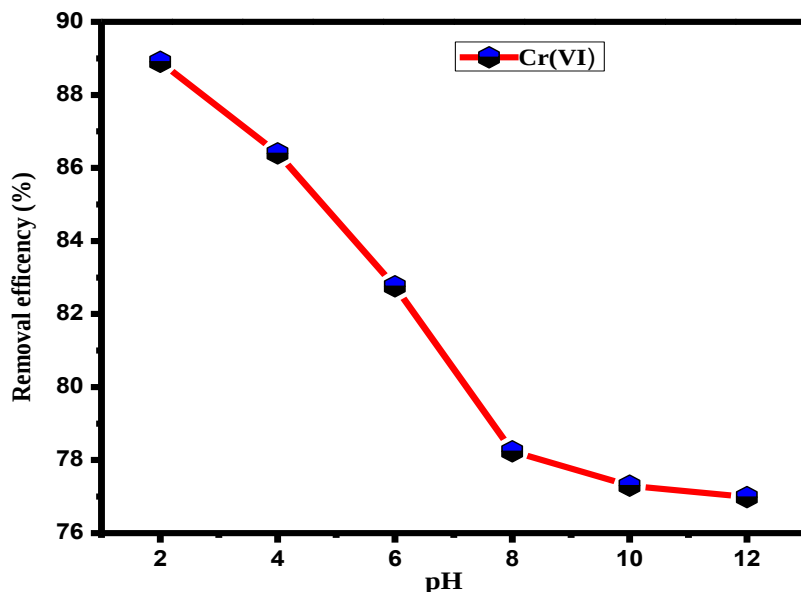


Figure 14: Effect of pH on the adsorption of Cr (VI).

4.4.2. Adsorbent Dosage

The adsorbent dose is an important parameter in adsorption studies because it determines the capacity of the adsorbent for a given initial concentration of Cr (VI) ions solution. In this study, the effect of adsorbent dose was determined by varying the amount of adsorbent, which ranged from 0.25 to 1.25 g and the results are shown in Figure 15. The Cr (VI) removal increases when adsorbent dosage increases, due to the increase of surface area available of $\text{Co}_3\text{O}_4(1:1)$ nano adsorbent for adsorption and the maximum removal efficiency of Cr (VI) was 90.1%. During optimization of adsorbent dose, the other parameters were fixed: 20 mg/L of initial concentration of chromium, solution pH of 2, 60 min contact time at 300 rpm agitation speed, and room temperature. Cr (VI) ions quickly adsorbed in the initial stage up to 1g and, then the removal efficiency decreased up on increasing the amount of the adsorbent. After that, the adsorbent surface and solution of Cr (VI) concentration reached equilibrium, and the removal efficiency was decreased due to a decrease in the active site of adsorbent. This result agrees with the previous literature report (Chakraborty et al., 2022). An adsorbent concentration of 1g of Co_3O_4 nano-adsorbent was then found to be the optimum value, where maximum Cr (VI) adsorption takes place.

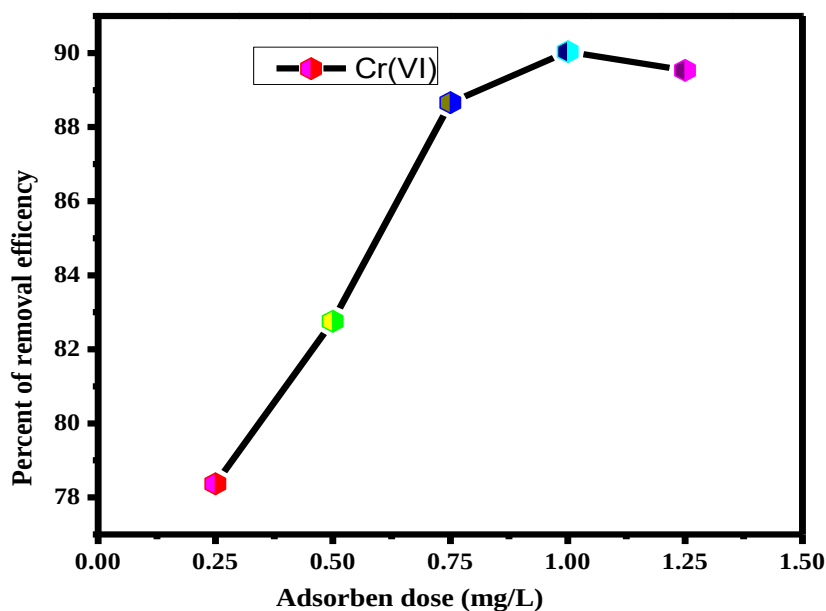


Figure 15: Effect of adsorbent dosage of Co_3O_4 NPs for adsorption of Cr (VI).

4.4.3. Effect of Initial Concentration of adsorbate

The effect of initial Cr (VI) concentration on the removal efficiency of Cr (VI) ion in aqueous solution was studied under the optimum condition (pH =2 adsorbent dosage 1g, contact time 60 min at 300 rpm agitation speed) and at room temperature. The effect of initial concentration was investigated in the range of (10- 50 mg/L). The observed results were shown in Figure16. The rate of percentage removal of Cr (VI) ion was high due to the large surface area of the adsorbent. Later, as a thick molecular layer was formed on the adsorbent, the adsorption capacity decreased (Chakraborty et al., 2022). The initial Cr(VI) concentration determines the equilibrium concentration and removal efficiency of ions. From the observed result when the initial Cr (VI) concentration varied from 10 to 50 mg/L, then, the removal efficiency of Cr (VI) on the surface of Co_3O_4 nano adsorbent decreased from 93.1% to 81%, and the optimum initial concentration was found to be 20 mg/L. Therefore, the result is in agreement with the previous literature reports (and Jain & Agarwal, 2020) and also, states the initial concentration of Cr (VI) ion increases, the active sorption sites are not sufficiently available on a fixed dosage of adsorbent and all the active sites on the adsorbent surface gate occupied, for this reason, the removal efficiency decrease. The adsorption efficiency of nano-adsorbent was found to be 93.1 % and the optimum value was at 20 mg/L of initial concentration of Cr (VI).

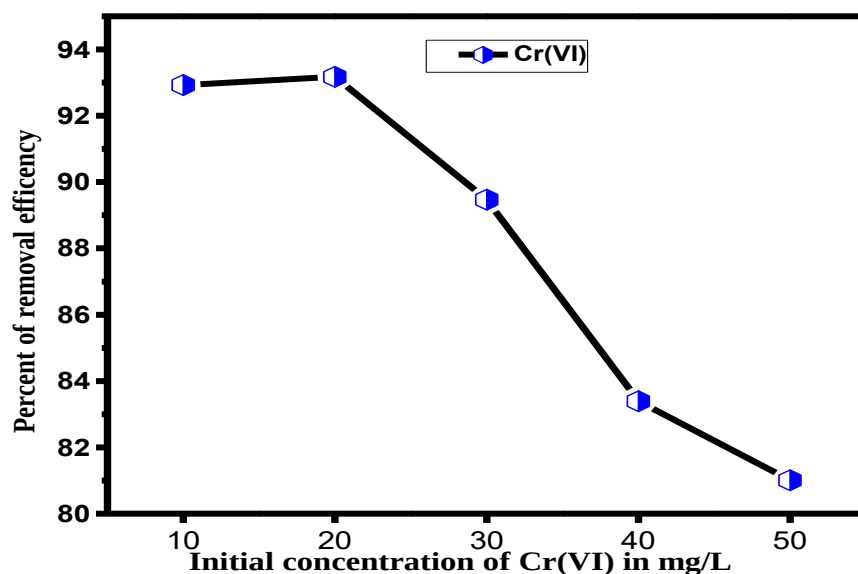


Figure 16: Effect of initial concentration of adsorbate on adsorption of Cr (VI).

4.4.4. Effect of Contact Time

The adsorption capacity and percentage removal of Cr (VI) by synthesized Co_3O_4 (1:1) nano adsorbent were optimized at the same initial concentrations (20 mg/L) the adsorbent dose of 1g and at a solution pH of 2. The effects of contact time for the removal of Cr (VI) ions from an aqueous solution were conducted by varying the contact times from 30-150 min and the agitation speed of 300 rpm at room temperature. The increments of contact or agitation time of the solution was also, increasing percent of removal efficiency and adsorption capacity of Cr (VI) because of the available adsorbing sites on the surface of the adsorbent increase (Kara et al., 2020). Figure 17, shows the effect of contact time on the adsorption of Cr (VI) on Co_3O_4 nano adsorbent for up to 150min. The observed result shows that Cr (VI) was quickly adsorbed in the initial stages due to the large adsorbent surface area. The amount of Cr (VI) removed slowly increased up to the equilibrium time obtained at 120 minutes but, after equilibrium, the adsorption process slowed down due to electrostatic repulsion between the adsorbent surface and Cr (VI) ions. The maximum adsorption efficiency of Co_3O_4 nano-adsorbent (1:1) volume ratio was 95.1%, at 120min. In addition to this (Jain & Agarwal, 2020) described a large number of vacant surface sites are available for adsorption at the equilibrium stage, and after a lapse of the availability of adsorbent sites the removal efficiency also slightly decreased because of the presence of fresh surfaces available in large time, the remaining vacant surface sites are difficult to be occupied due to repulsive force between the solute molecules on the solid. The number of vacant binding sites declines and becomes tricky to

use because of the creation of electrostatic repulsion among residual Cr (VI) ions in the solution.

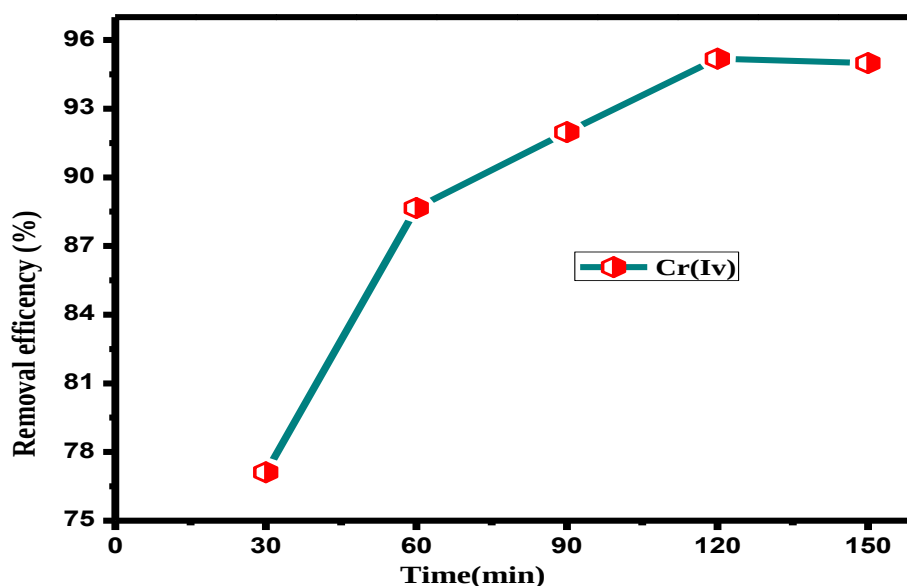


Figure 17: Effect of contact time on the adsorption of Cr (VI).

4.5. Adsorption Isotherms

The isotherm provides information to study the relationship between the adsorbate and adsorbent concentration at equilibrium and the maximum adsorption capacity of adsorbent at a constant temperature or explained the adsorption process and gave information on the adsorbent capacity and surface properties (Soliman & Moustafa, 2020b). The relationship between the concentration of Cr (VI) in solution and the amount of chromium adsorbed on the solid phase adsorbent at equilibrium was explained. The most widely used isotherm models are Langmuir and Freundlich's equations. These isotherms are critical for the optimization of the adsorption mechanism and the loading capacity of the adsorbent (Samuel et al., 2021).

4.5.1. Langmuir isotherm model

The Langmuir isotherm is the most widely used model and is based on the assumption that monolayer coverage occurs at specific homogeneous sites on the surface of the adsorbent having a constant number of identical and energetically equivalent adsorption sites without any departure into the plane of the adsorbent surface (Chakraborty et al., 2022). Once a site is filled, no further adsorption can take place at that site. This indicates without any interaction between the adsorbate on the adjacent site and the surface reaches a saturation point where the maximum adsorption of the surface will be achieved. The linear form of the Langmuir isotherm model is given by

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \dots\dots\dots 6$$

Where q_e is the amount of Cr (VI) adsorbed per amount of adsorbent (mg/g) at equilibrium, q_m is the maximum adsorption capacity (mg/g), K_L is the Langmuir isotherm constant (L/mg), and C_e is the equilibrium concentration of Cr (VI) in solution (mg/L). Langmuir constants, which are related to the adsorption capacity (q_m) and energy of adsorption (k_L), can be calculated from the slope of the linear plot of C_e/q_e v/s C_e ; a straight line with slope $1/q_{\max}$ and intercept of $1/q_{\max} k_L$ is obtained. The essential characteristics of the Langmuir equation can be expressed in terms of a dimensionless factor (R_L), and also the feasibility of the adsorption process is expressed. The separation factor (R_L) can be calculated by the equation.

$$R_L = \frac{1}{1 + K_L C_0} \dots\dots\dots 7$$

Where C_0 is the highest initial Cr (VI) in (mg/L), the values of the separation factor R_L have their usual significance. R_L value indicates that the isotherm and the adsorption process are either unfavorable if ($R_L > 1$), linear if $R_L = 1$, or favorable if $0 < R_L < 1$. Then in this study from Table 7 R_L value is 0.041 which means the value of R_L is between 0 and 1 it indicates the adsorption of chromium on the surface of Co_3O_4 NPs is favorable. Figure 18 shows the equilibrium isotherms for the adsorption of chromium on Co_3O_4 (1:1) NPs, and the equilibrium adsorption data were analyzed by using the Langmuir isothermal model.

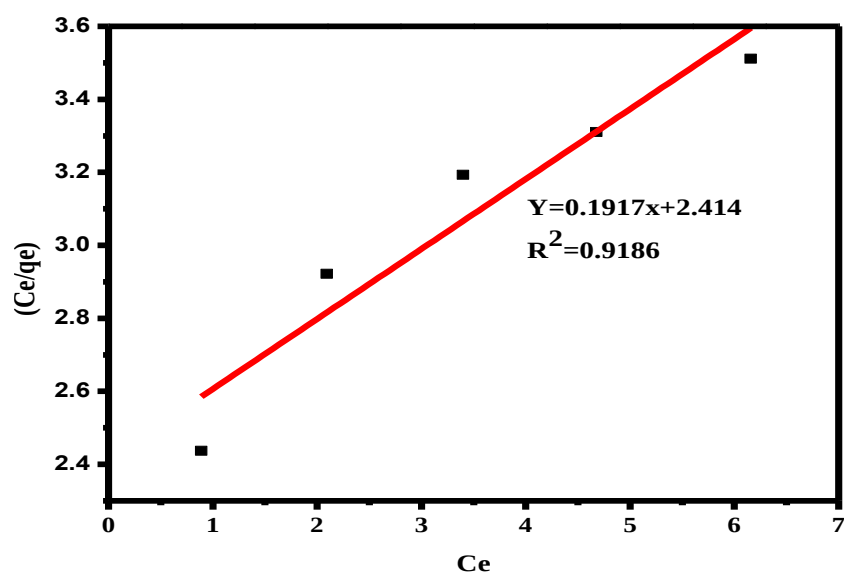


Figure 18: Langmuir adsorption isotherm model.

Table 7: Langmuir adsorption isotherm parameter

Langmuir isothermal model	Parameter	value
	$K_L=1/q_m*\text{intercept}$	0.46276
	$q_{\max}=1/\text{slope}$	5.2164
	$R_L=1/(1+k_L*Co)$	0.04142
	R^2	0.9186

4.5.2. Freundlich isothermal model

Freundlich's isothermal model depends on the assumption that the adsorption of metal ions on the adsorbent takes place by multilayer adsorption on a heterogeneous surface, with the interaction between the adsorbed molecules. For the Freundlich isotherm if the value of n (g/L) is an indication of the adsorption deviation from linearity and the value of $1/n$ is the heterogeneity factor. A value of n between 1 and 10 (i.e., $1/n < 1$) shows favorable adsorption. For $n=1$, linear adsorption is found; but for $n<1$, adsorption becomes a chemical process; while for $n>1$, adsorption is a physical process and also, $n=1$ then the partition between the two phases is independent of the concentration. If the value of $1/n$ is below one it indicates normal adsorption. On the other hand, $1/n$ being above one indicates cooperative adsorption. The linear form Freundlich isotherm can be represented by

$$\text{Log } q_e = \text{Log } K_f + \frac{1}{n} \log C_e \dots\dots\dots 8$$

Where, q_e is the amount adsorbed at equilibrium (mg/g), K_f and n are the Freundlich constant, $1/n$ is the heterogeneity factor which is related to the capacity and intensity of the adsorption, and C_e is the equilibrium concentration (mg L^{-1}). The values of K_f and $1/n$ can be obtained from the slope and intercept of the plot of $\log q_e$ against $\log C_e$. For this study as shown in Table 7 the value of $1/n$ was below 1 ($1/n=0.81$) for all adsorbents which indicates the normal adsorption. Figure 19 shows the equilibrium isotherms for the adsorption of chromium on Co_3O_4 (1:1), nanoparticles, and, the equilibrium adsorption data were analyzed by using Freundlich isotherm models.

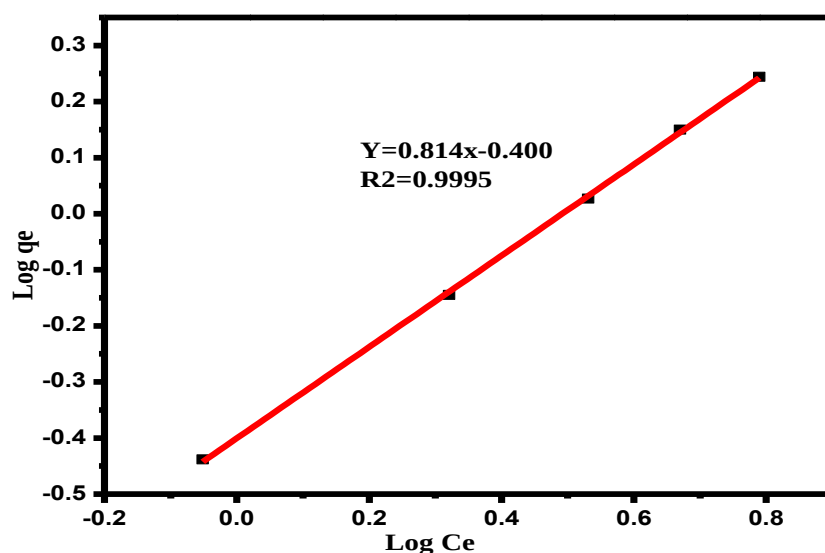


Figure 19: Freundlich Adsorption Isotherm.

Table 8: Freundlich Adsorption Isotherm parameters

Freundlich isothermal model	Parameter	Value
	Kf =antilog of intercept value	0.3981
	n=1/slope	1.2285
	R ²	0.9995

In general, based on the above experimental graph, the R^2 values of the Langmuir model are less than Freundlich models for Co_3O_4 (1:1) nano-adsorbent this indicates that the Freundlich model is suitable for describing the adsorption equilibrium of Cr(VI) on the surface of Co_3O_4 nano adsorbent.

4.6. Kinetic Study

Kinetic models have been exploited to test the experimental data and to find the mechanism of adsorption and its potential rate-controlling step that include mass transport and chemical reaction. The adsorption mechanism depends on the physical and chemical properties of the adsorbent. The mechanism of the rate control of the adsorption process depends on diffusion, chemical reaction, and mass transfer (Chakraborty et al., 2022). The study of the adsorption kinetics describes the rate of the adsorbate uptake on the adsorbent at different time intervals. The kinetic parameters were mostly analyzed by pseudo-first-order and pseudo-second-order models (Jain & Agarwal, 2020).

4.6.1. Pseudo First Order Kinetics Model

The Pseudo-first-order model assumed that the rate of adsorption is proportional to the number of free sites model and to determine if the adsorption process fits with its model or not. The experimental data were done at different concentrations (40 mL of 10-50 mg/L and 1g of Co_3O_4 nano- adsorbent dosage) the pH of 2 and contact time (30-150 min) at room temperature and shaker speed of 300rpm. The rate constant and q_e were calculated from the slope and intercepts of the graph $\log(q_e - q_t)$ vs. t graph stated in equation 9.

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \dots \dots \dots 9$$

Where q_e and q_t represent the amount of adsorbed (mg/g) at equilibrium and at any time t and k_1 are the first-order rate constant (min^{-1}), from the plots of $\log(q_e - q_t)$ versus t in Figure 20, k_1 can be calculated from the slope and theoretical q_e can be obtained from intercepts. From the graph, the coefficient of determination value (R^2) of adsorption of Cr (VI) on the surface of Co_3O_4 nano-adsorbent was $R^2 = 0.8538$.

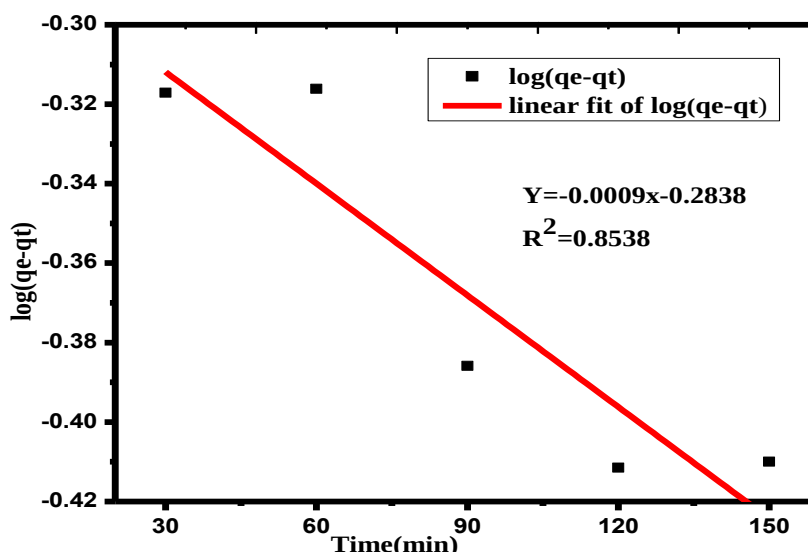


Figure 20: Pseudo first-order kinetic curve.

4.6.2. Pseudo Second-Order Kinetic Model

To determine whether the adsorption mechanism is determined by pseudo-second-order or not the graph of t/q_t vs. t was plotted using as stated in equation 11. The linearity was observed by considering the correlation coefficient value. The values of q_e and K_2 were calculated from the slope and intercept of the plot of $(t/q_t$ vs. $t)$ respectively and Table 9 summarizes the value of

kinetics parameters. The pseudo-second-order plot of Cr (VI) adsorption on the surface of Co_3O_4 nano-adsorbent for different concentrations was shown in Figure 21. The initial concentration of chromium was (10-50 mg/L) and contact time was 30-150min the other parameter were constant such as pH 2 and adsorbent dosage of 1g at room temperature. In this work the coefficient of determination (R^2) value is 0.994 then the value of R^2 for the pseudo-second-order was higher compared to the R^2 value of the pseudo-first-order. Then higher R^2 value obtained indicated the adsorption of Cr (VI) ion on Co_3O_4 nano-adsorbent was fitted with a pseudo-second-order kinetics model.

$$\frac{t}{qt} = \frac{1}{K_2 q_e^2} + \frac{1t}{q_e} \dots\dots\dots 10$$

Where K_2 is the adsorption rate constant (g/mg-min), The K_2 and q_e are found from the intercept and slope of (t/qt versus t) linear plot such that $1/q_e$ as a slope and $1/k_2 q_e^2$ as intercept.

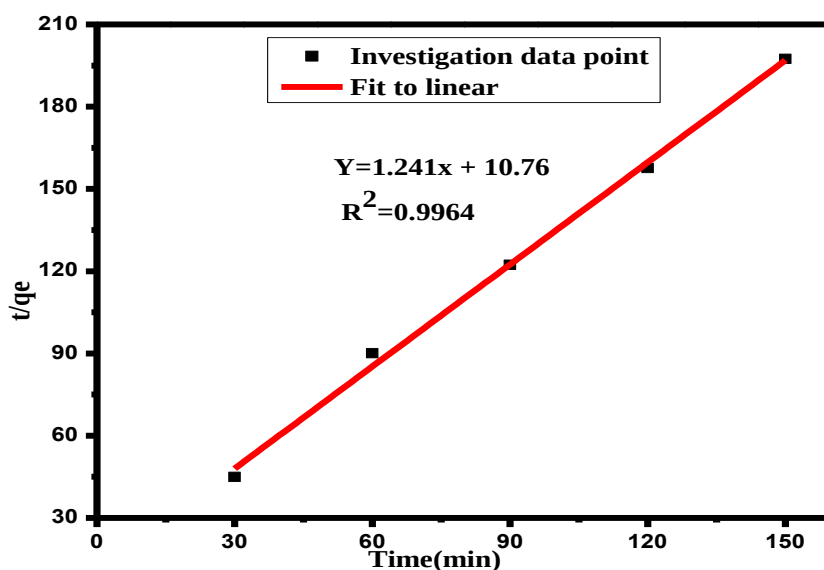


Figure 21: Pseudo second order kinetics curve.

Table 9: Linear equations of the pseudo-first and pseudo-second order equation

Pseudo first order					
Slop	intercept	R ²	qe=antilog of intercept	K ₁ =2.303*slop	
-0.0009	-0.2838	0.8538	0.520235	0.00207	
Pseudo second order					
Slop	intercept	R ²	qe ² =0.73*qe	qe= 1/slop	K ₂ =1/intercept*qe ²
1.241	10.76	0.9964	0.600236	0.805802	0.055784

4.7. Antibacterial Activity of Cobalt Oxide Nano-drug

Antibacterial activity of synthesized Co_3O_4 nano-drug in three volume ratios from *Ananas comosus* peel extracts were investigated and compared with the positive control Ampicillin by using the disc diffusion method. To confirm the antibacterial capacity, *P. aeruginosa* ATCC27853, *E. coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and, *S. pyogen* ATCC19615 have been used. The antibacterial activity of Co_3O_4 nano-drug increases with increase the concentration of prepared solution in three different amounts in $\mu g/mL$ (50, 75, and 100) and observed in the bottom. The result of the study was clearly described as shown in Figure 22 and supported by Table 10.

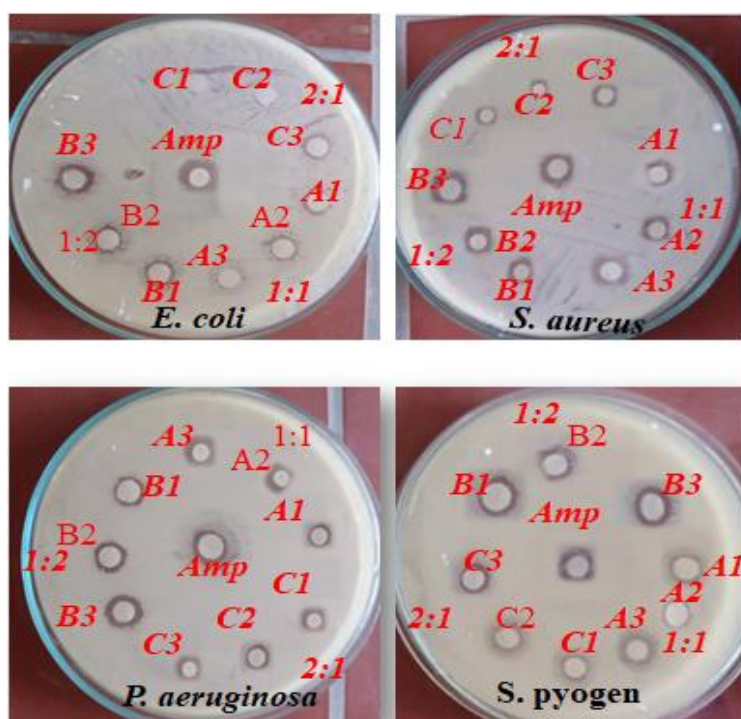


Figure 22: The zone of inhibition of synthesized Co_3O_4 (1:1, 1:2, and 2:1 ratio) nano-drugs against *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. pyogenes* at 50, 75, and 100 $\mu g/mL$ concentration.

Table 10: Zone of inhibition of Co₃O₄ nano-drug against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. pyogen*

Ratios	Conc. (Co ₃ O ₄ NPs)	Bacteria Species and Zone of Inhibition			
		<i>E. coli</i> ATCC25922	<i>S. aureus</i> ATCC25923	<i>P. aeruginosa</i> ATCC27853	<i>S. pyogen</i> ATCC19615
1:1	A1= 50 µg/mL	8	9	7	8
	A2= 75 µg/mL	9	10	9	10
	A3= 100 µg/mL	10	11	10	11
1:2	B1= 50 µg/mL	9	9	9	10
	B2= 75 µg/mL	11	11	10	12
	B3= 100 µg/mL	12	13	12	13
2:1	C1= 50 µg/ml	7	8	7	8
	C2= 75 µg/ml	7	8	8	9
	C3= 100 µg/ml	8	9	8.5	10
Ampicillin (+ve control)		13	13.5	13	13

The antibacterial activity of biological synthesized Co₃O₄ nano-drug in various volume ratios was determined each after one day of the experiment using different doses (50,75 and 100 µg/mL) shown in Figure 22 and Table 10. Almost all strains were found susceptible to Co₃O₄ nano-drug. However, in this study, *Pseudomonas aeruginosa* was the least susceptible strain relative to the other, and *S. pyogen* was the most susceptible strain by Co₃O₄ nanodrug. The antibacterial activity of Co₃O₄ nano-drug in (1:1) (1:2) and (2:1) by volume ratios was found in the range of (8-11) (9-13) and (7-10) mm respectively against *E.coli*, *S.aureus*, *P.aeruginosa*, and *S. pyogen*. Depending on the result, antibacterial activity of synthesized Co₃O₄ nano-drug in three volume ratio was found to increase within increase the amount of peel extracts as shown in Figure 21 and Table 10. Biological synthesized Co₃O₄ (1:2) nano-drug was found to be the most effective antibiotic activity as compared to its counterparts. It indicates that, might be high antibacterial performance and to contribute high ROS production. Then the high ROS production results from the use of the large volume of *Ananas comosus* peel extract which contains amounts of OH radical. On the other hand Co₃O₄ (1:1) nano-drug has shown good performance followed by the Co₃O₄ (1:2) ratio, due to the exit of the bioactive compound within an equal volume ratio of metal salt and interaction with the surface of the bacteria cell wall and to penetrate it. However, the (2:1) volume ratio of Co₃O₄ nanodrugs showed little performance as compared to the other ratios. The concentration of Co₃O₄ nanodrug was increased it has a better antibacterial activity or has a good inhibition zone for both Gram-positive and Gram-negative bacteria. Gram-positive *Streptococcus pyogenes*, and

Stybloccocos aurous bacteria were equal inhibition zone with positive control Ampicillin and 100 $\mu\text{g/mL}$ concentration of Co_3O_4 (1:2) nano-drug. From the result, it was observed that Gram-positive bacteria were more affected by high concentrations of Co_3O_4 nano-drugs. In aqueous solutions of Co_3O_4 nano-drugs, reactive species such as cobalt cations, superoxide radical anions, and hydroxyl radicals develop. Co^{++} released by cobalt oxides interact with the thiol group of key bacterial enzymes, causing inactivation and cell death, or radicals present in aqueous Co_3O_4 nano-drug interact with the negatively charged bacterial cell surface, interfering with vital life functions like breathing and cell reproduction (Haq et al., 2021). Generally, Gram-positive bacteria were more inhibited by nanodrugs than Gram-negative bacteria because it is a thick bacteria cell wall formed from polysaccharides and simply penetrated by antibiotics and Gram-negative bacteria can resist nano-drugs in case of the cell wall of such bacteria were covered by lipoprotein and can resist antibiotics (Hafeez et al., 2020).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this study, Co_3O_4 NPs were biologically synthesized from cobalt nitrate hexahydrate and *ananas comosus peel extract* within 1:1, 1:2, and 2:1 volume ratios. The synthesized Co_3O_4 NPs were characterized using TGA-TDA, XRD, SEM-EDS, UV-DRS, FTIR, and, BET techniques. Based on the TGA-DTA analysis the synthesized Co_3O_4 (1:1) NPs were found to be thermally stable above 500°C and XRD analysis reveals the average crystallite sizes of 12.58, 13.40, and 22.05 nm for the volume ratios of 1:2, 1:1, and 2:1 Co_3O_4 NPs, respectively. SEM-EDS indicate the spherical morphology of Co_3O_4 NPs was synthesized without impurity. UV-DRS result suggests the energy band gap of Co_3O_4 NPs synthesized with the volume ratios of 1:1, 1:2, and 2:1 were conducted to be 3.33, 2.95, and 2.70 eV, respectively and the specific surface area of each Co_3O_4 NPs were 24.7, 23.7 and $22.50 \text{ m}^2/\text{g}$ determined using BET analysis. In addition, the FTIR analysis confirms the role of peel extract of as *Ananas comosus* and Co-O interaction (stretching) in synthesized Co_3O_4 NPs. Moreover, the synthesized Co_3O_4 (1:1) NPs were used as an effective adsorbent for the removal of Cr(VI) ions in an aqueous solution due to the presence of a small surface area and particle size. The maximum removal efficiency of Cr (VI) was 95% by using 1g Co_3O_4 (1:1) NPs, at 2 pH 20 mg/L initial concentration and 120 min contact time at room temperature. The equilibrium data were better described by the Freundlich isotherm model and the adsorption kinetics was found to fit the pseudo 2nd order kinetic model within this work. Finally, the antibacterial activity of synthesized Co_3O_4 NPs in 1:1, 1:2, and 2:1 by volume ratios was evaluated against *S. pyogen*, *S. aureus*, *P. aeruginosa*, and *E. coli*. Among the three volume ratios of Co_3O_4 NPs, 1:2 nano-drugs was a better potential for antibacterial activity compared to the counterparts and showed enhanced antibacterial activity (13 mm) towards *S. aureus* and *S. pyogen*. Due to the presence of a high concentration of peel extract which consists of bioactive compounds attributed to the formation of enhanced reactive oxygen species (ROS) and interaction with the bacterial cell wall and death. Gram-positive bacteria were more affected by Co_3O_4 nano-drug than Gram-negative bacteria.

5.2. Recommendations

Green synthesized cobalt oxide NPs could be tested for the removal of toxic and heavy metal ions from industries and real waste/sludge/.To enhance the adsorption efficiency of green synthesized cobalt oxide NPs, several protocols such as nanocomposite, core-shell, dope, and coated methods will be investigated. The antibacterial activity of green synthesized cobalt oxide NPs could be enhanced it will be done in the presence of UV light, and also to apply practically, researchers may include health center staff such as doctors, pharmacies, and other research institutions. The chemical composition and oxidation state of the synthesized Co_3O_4 NPs should be characterized by X-ray photoelectron spectroscopy (XPS). It is also possible to study the reusability of synthesized Co_3O_4 nano-adsorbent for the removal hexavalent chromium ion.

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