Chemical and Biological Synthesis of Cobalt Oxide Nanoparticles: Comparative Study of Antibacterial Activity

By: Abu Tollosa

A Thesis Submitted to the Applied Chemistry Department

School of Applied Natural Science

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Physical Stream

Office of Graduate Studies

Adama Science and Technology University

July, 2019

Adama, Ethiopia

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Major advisor: Fedlu Kedir (PhD)

Co-advisor: Gemechu Deressa (PhD)

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Approval Sheet

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As thesis research advisors, We hereby certify that we have read and evaluated this thesis prepared under our guidance by Abu Tollosa. We recommended the paper to be submitted as fulfilling the requirements for the Degree of Master of Science in Chemistry (Physical Chemistry).

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We the undersigned members of the boards of examiners of the final open defence by **Abu Tollosa** We have read and evaluated his thesis entitled **"Chemical and Biological Synthesis of Cobalt Oxide Nanoparticles: Comparative Study of Antibacterial Activity"** This is therefore to certify his thesis work and has been accepted in partial fulfillment of the requirements of the degree of **Master of Science in Chemistry (Physical Chemistry)** Complies with the regulation of The University.

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DECLARATION

I hereby declare that the thesis entitled "Chemical and Biological Synthesis of Cobalt Oxide Nanoparticles: Comparative Study of Antibacterial Activity" has been carried out by me under the supervision of Dr. Fedlu Kedir and Dr.Gemechu Deressa, during the year 2019 as part of Master of Science Program in Physical Chemistry. I further declare that this work has not been submitted to any other Universities or institutions for the award of any degree. All quotations and their sources are specifically acknowledged by means of references.

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DEDICATION

This M.Sc thesis is dedicated to my beloved families, especially to my Father Tollosa Adino and to my wife Tanagne chelkeba whose advice, support, prayers and love helped me all along the right way and made me who I am today. They will always be in my heart forever.

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LIST OF ABBREVIATIONS AND ACRONYMS

FCC	Face centered cubic
E.coli	Escherichia coli
FTIR	Furrier transform infrared
MIC	Minimum inhibitory concentration
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
S.aureus	staphylococcus aureus
UV-Vis	Ultra violet visible
XRD	X-ray Diffraction
NPs	Nanoparticles
MARSA	Methicillin resistant staphylococcus aureus
BCC	Body centered cubic
HCP	Hexagonal close packed
TB	Tuberculosis
DI	Deionized water
Eg	Bandgap energy
DRS	Diffused reflectance spectroscopy
GC	Green synthesized calcinated nanoparticles
UG	Uncalcinated green synthesized nanoparticles
EDX	Energy Dispersive x-ray spectroscopy

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ABSTRACT

Green synthesis of NPs using plant extract is a novel method to develop environmentally benign nanomaterials which can be used in numerous biomedical applications. In this study we have synthesized cobalt oxide nanoparticles through biological method using cobalt nitrate hexahydrate as a precursor and indigenous plant extract, Indod (Phytolacca dodecandra) leaf as a reducing and capping agent. Furthermore, the NPs were synthesized chemically by co-precipitation method from cobalt nitrate hexahydrated in the presence of sodium hydroxide as a precipitating agent. The synthesized nanoparticles were characterized using X-Ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. The crystal size of the synthesized NPs was calculated using Debye-Scherer equation and was found to be 10.79 nm and 11.9 nm for biologically and chemically synthesized Co_3O_4 nanoparticles, respectively. The bandgap for biologically and chemically synthesized nanoparticle (NPs) were 3.35 eV and 3.2 eV, respectively. The XRD pattern with sharp peaks describes the crystalline and purity of cobalt oxide nanoparticles. The shape and morphology of Co_3O_4 nanoparticles were studied by SEM analysis confirms spherical shape and EDX also gave the percent composition of the atom. Thus elemental composition of Co and O in synthesized nanoparticle was 73.22, 94.92% for green and chemically synthesized Co_3O_4 NPs, respectively. FTIR spectrum discloses the information about the interaction between the functional groups of the phytochemical in the leaf extract and the Co_3O_4 NPs. Based on the results biologically synthesized Co_3O_4 nanoparticles calcinated and Uncalcinated have potential antibacterial applications even in small concentration specially for gram negative bacteria compared to chemically synthesized Co_3O_4 nanoparticles.

Key word: Co_3O_4 *nanoparticles*, *Green synthesis, Co-precipitation, Antibacterial activities.*

1. INTRODUCTION

1.1. Background of the study

Nanotechnology deals with the production and design of utilizing nanoparticles. It emerges from the physical and chemical properties different from bulk and engineered techniques are being developed to search and plan single atoms and molecules for multiple applications in different field of scientific world. Nanoparticle (NP) is defined as a small object that behaves as a whole unit in terms of its properties. NPs are cluster of atoms having at least one dimension in the size range of 1–100 nm. They have unique optical, magnetic, catalytic and electrical properties. Thus have potential applications in various fields the physicochemical properties of NPs are different as compared to those of their bulk counterparts owing to the fact that surface area to volume ratio increases and quantum effects become dominant as the size decreases. The increase in surface area to volume ratio alters the mechanical, catalytic and thermal properties of material (DeSimone, 2002).

The application of nano technological products in human activities has been rapidly increasing in the past decade largely due to the unique properties of engineered nanoparticles. Almost interests are metal nanoparticles and their biological effects since these nanoparticles have been widely used in cosmetics, medicine, electronics and industry. Cobalt is a transition and magnetic metal and has special characteristics such as high surface area due to their small size high magnetism and unique catalytic properties. It is widely used in industrial applications such as magnetic tape, chemical catalysis, gas sensing equipment, coating, and light absorbance as well as in medical biotechnology such as magnetic resonance imaging (Bouchard *et* al., 2009).

Due to the widespread applications of nanoparticles in different technology and biotechnological aspects the preparation of different kinds of nanoparticles became very important nowadays (Ma'mani *et* al., 2010). A nanoparticle with various compositions is prepared by physical, chemical and biological methods. The physical methods include evaporation, sputtering, laser ablation, ion ejection, and electron- beam lithography. The chemical methods include salt reduction method, reverse micelles, electrochemical method, Sol-gel method, gas-liquid interface, thermolysis, co-precipitation and decomposition on ultrasonic treatment. Green chemistry and biological processes has led to develop an environment-friendly process for the synthesis of nanoparticles. A vast array of biological resources available in nature including living plants, plant products, and plant extracts, Algae, fungi, yeast, bacteria and viruses could all be employed for synthesis of nanomaterial (Gubin *et* al., 2005).

One of the global health and environmental concerns is the increasing antimicrobial resistance of many micro organisms against drugs because some pathogens which were curable in past now are becoming untreatable such as Methicillin-resistant staphylococcus aureus (MRSA) (Kitakami *et* al.,1997). Humans have always warand struggle against the microorganisms that cause infection and disease to Human health. To win this battle and to overcome this frightening situation of microbial resistance to antibiotics the search and discovery of alternative means against these microorganisms is only way forward to survive. Metals based nanoparticles and investigation of their antimicrobial activity could be one of such alternatives. Many studies are being done to check the structural and chemical effects of various metals nano-complexes to discover an alternative of the drugs which used against microorganisms. Cobalt oxide nanoparticles is also among those materials which can be used for antimicrobial activity so the synthesis, characterization and antimicrobial activities of Cobalt based nanoparticles of great importance.(Sekhon, 2010).

1.2. Statement of the problem

Bacterial infectious diseases are serious health problem that has drawn the public attention in worldwide human health threat which extends to economic and social complications. Outbreaks infections of pathogenic strains, bacterial antibiotic-resistance, emergence of new bacterial mutations, lack of suitable vaccine is a reason why infection due to pathogen is increased (Liao *et* al.,2011). In under developed countries hospital-associated infections are global health hazard to human particularly in children. Thus developing novel antibacterial agents against bacteria strains mostly major pathogens such as *Escherichia coli* and *staphylococcus aureus* has become greatest demand. These two bacterial strains are selected as they are highly transmittable hence we can evaluate the potential antimicrobial activity of cobalt oxide nanoparticles against these pathogenic bacteria. Different researches have done on the synthesis of Cobalt Oxide nanoparticles for antibacterial applications however; synthesis of nanoparticles by green methods using indigenous plant extracts is not widely done. Furthermore, comparative study on Co_3O_4

NPs synthesized by chemical using cobalt nitrate hexahydrate and green method using *Phytolacca dodecandra* plant extract as reducing agent *against* human pathogenic bacteria has not been reported yet. The present study aims at preparation of cobalt oxide NPs in chemical and green method using the plant extract of *Phytolacca dodecandra* and studying its application for antibacterial activity and comparing the efficiency of cobalt oxide NPs prepared using these two methods.

1.3. Objectives of the Study

1.3.1. General Objective

The general objective of this project was to synthesize cobalt oxide nanoparticles by chemical method using co-precipitation and green method using leaf extract of *Phytolacca dodecandra* and investigate their antibacterial application

1.3.2. Specific Objectives

- To synthesize Co_3O_4 NPs using leaf extract of *Phytolaccadodecandra* (Indod) and cobalt nitrate hexahydrate.
- To synthesis Co_3O_4 NPs by chemical method using co-precipitation from cobalt nitrate hexahydrate.
- To characterize the synthesized cobalt oxide nanoparticles using UV-Vis, FTIR, SEM-EDS, and XRD.
- To compare antibacterial Activity of biosynthesized cobalt oxide NPs obtained from and chemically synthesized against human pathogenic bacteria such as *E.coli* and *staphylococcus aureus.*

1.4. Significance of the Study

The study area was provided important information about the biologically and chemically synthesized cobalt oxide nanoparticles and characterization methods. This study area was also gave information about antibacterial activities of cobalt oxide nanoparticles synthesized using chemical and biological methods. Also opens up a door for further explorations on many of Ethiopian indigenous plants used for antibacterial applications.

1.5. Scope of the Study

Biosynthesis of cobalt oxide nanoparticles using *Phytolaccadodecandra leaf* extract as a reducing agent and chemically synthesized cobalt oxide nanoparticle by co-precipitation method were characterized using instruments such as UV-Vis, FTIR, SEM-EDS and XRD. Finally antibacterial activities were evaluated for Co_3O_4 NPs synthesized using both methods.

2. LITERATURE REVIEW

2.1. Nanoparticles

Nanotechnology refers to the production and usage of material with nanoscale dimension. It is a multidisciplinary scientific field undergoing explosive development and one of the most active research areas in modern material science. Because of Specific characteristics such as size distribution and morphology nanoparticles exhibit completely new or improved property. NPs have given rise to a busy area of research due to the wide variety of applications and novel properties such as biomedicine and with tunable electrical conductivity, thermal conductivity, superior rigidity, hard and erosion resistance. The metallic oxide nanoparticles are most promising which is of much interest to researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Issa *et* al., 2013).

2.2. Synthesis of Metal Oxide nanoparticles

The methods for the synthesis of nanoparticles are physical, chemical and biological techniques Physical synthetic methods such as inert gas condensation, high energy ball milling and ultrasonic shot peeling can be used to synthesize metallic nanoparticles (Li & Zhang, 2006). Several methods including the commonly grinding process and pyrolysis can be used for the physical synthesis of metallic oxide nanoparticles. Disadvantage of physical methods is expensive and cumbersome for the large-scale production of nanoparticles (Duan *et* al.,1999). A further drawback of physical approaches is enormous consumption of energy to maintain the high pressure and temperature used in the synthesis procedures.

Chemical methods involve the reduction of chemicals, electrochemical procedures, micro emulsion, chemical co-precipitation and chemical vapor condensation. A typical procedure involves growing nanoparticles in a liquid medium containing various reactants in particular reducing agents such as sodium borohydride or potassium bitartrate polyethylene glycol or hydrazine (Guzmán *et* al., 2009).

Biological synthesis method of NPs which has the advantages of no toxicity, reproducibility in production easy scaling-up has become a new trend in nanoparticle production. Particular microorganisms and plants have been demonstrated as new resources with considerable potential for synthesizing nanoparticles (Sorbiun *et* al., 2018). In the case of biological method NPs synthesis using plant extracts is the most adopted method because it is eco-friendly. The green method production of nanoparticles is much safer to handle and easily available (Ankamwar *et* al.,2005). While fungi and bacteria require a comparatively longer incubation time. Plant based synthesis of NPs works at low temperatures and requires only modest and environmentally safe components (Abdel-Halim *et* al., 2011). In addition the synthesis of nanoparticles using plants offers other advantages such as the utilization of safer solvents decreased use of dangerous reagents, milder response conditions, feasibility and their adaptability in use for medicinal, surgical and pharmaceutical applications. Also physical requirements for their synthesis including pressure, energy, temperature and constituent materials are trivial (Ledwith *et* al., 2007).

In the biosynthesis of metallic oxide nanoparticles using plant extract three important parameters are needed metal salt, a reducing agent and a stabilizing or capping agent for controlling the size of nanoparticles and preventing their aggregation. Many biomolecules in plants such as proteins, amino acids, carbohydrates, alkaloids, phenol compounds, reducing sugar and vitamins could be involved in bioreduction, formation and stabilization of metal nanoparticles. The reduction potential of ions and reducing capacity of plants which depend on the presence of polyphenols, enzymes and other chelating agent present in plants have critical effects on the amounts of nanoparticle production (Morones et al., 2005).

2.2.1. Cobalt Oxide Nanoparticles

Cobalt has been known with allotropic forms including face centered cubic (FCC), Hexagonal centered phase (HCP), epsilon and body centered cubic (BCC) The FCC structure is thermodynamically preferred at higher temperatures and the HCP structure is favored at lower temperatures. The FCC structure appears to be stable phase even below room temperature when the particle sizes are smaller than 20nm (Löffler *et* al., 2001). There has been a considerable amount of research involving the preparation, structure and properties of magnetic cobalt nanoparticles in the past decade (Zhang *et* al., 2000). The high magneto crystalline anisotropy of HCP Cobalt has spurred intensive studies of Cobalt based nanostructures for magnetic storage purposes. Cobalt nanoparticles coated with insulators have been prepared and studied for the applications in AC electrical and electronic devices (Zhan *et* al., 2001).

Different crystalline structures provide some practical benefits. Symmetrically structured FCC cobalt provides higher saturation moment and lower magnetic anisotropy which is suitable for linear applications such as converter. On the other hand asymmetric HCP-cobalt has been a basic element for high temperature permanent magnets and suitable for microwave applications such as in circulator. Cobalt nanoparticles often possesses mixed structures in which low energy stacking faults introduce a combination of FCC and HCP character making the synthesis of single structured cobalt a challenging task. The potential applications of Cobalt oxide NPs in separation technology, catalysis, high-density magnetic recording and biomedicine are reviewed (Yu *et* al., 2015).

2.3. Biosynthesis of Co_3O_4 NPs

Extract from bio-organism may act both as reducing and capping agent in nanoparticles synthesis. The reduction of metals ions by bimolecular found in these extracts such as protein, polysaccharide, vitamin and amino acid is environmentally save. Biological methods are regarded as safe, cost effective, sustainable and environmental friendly processes for the synthesis of nanoparticles. Biological synthesis of nanoparticles is gaining importance due to its eco friendliness and simplicity (Biradar et al., 2012). The use of plant materials in the synthesis of cobalt oxide nanoparticles has been reported by other researchers. These plant materials include the leaves of *Conocarpus erectus,* leaves of *Raphanussativus var. longipinnatus* and dried powdered fruits of piper longum (Ahmed *et* al., 2016). Herein is reported the biofabrication of cobalt nanoparticles using leaf extract of *Chromolaenaodorata* and their antibacterial application.

2.4. Chemical Synthesis of Co_3O_4 nanoparticles

The transition metal oxides have greatly attracted wide range of application in durable solar absorber lithium-ion battery. As transition metal groups cobalt oxide Co_3O_4 NPs is one of the most important materials because of its fascinating properties and thermal stability. Co_3O_4 NPs show good conductivity due to the existence of Co^{3+} (Salavati *et* al., 2009). Co_3O_4 NPs synthesized by co-precipitation method is a simple process for the formation of nanoparticle and to control size of nanoparticles. Cobalt nanocrystals display a wealth of size-dependent structural magnetic, electronic and Catalytic properties. The cobalt structure of p-type semiconducting materials with chemical stability at high temperature high mechanical strength and bandgap 1.482.19eV (Kim et al., 2018). Reported synthesis of Co_3O_4 nanoparticles at 750 ^{0}C using sol-gel method (Yarestani *et* al., 2014). Cobalt oxide nanoparticles were prepared in aqueous KOH solution with hydrothermal method have been studied electrical conductivity at different temperature. Thus in the present day the stable phase of Co_3O_4 NPs is synthesized by Co-precipitation method without any capping agent (Makhlouf *et* al., 2013).

2.5. Antibacterial activity of metallic Oxide nanoparticles

Metal based nanoparticles represent an alternative for biomedical treatments mainly in the fabrication of biomedical devices with antimicrobial coatings. A high antimicrobial activity of nanoparticles depends on the particle size that allows greater surface contact and a direct interaction with the membranes microorganisms. Nanoparticles smaller than 10 nm highly interact with bacteria and produce electronic effects which improve the reactivity of nanoparticles. Thus it is proven that the bactericidal effects of nanoparticles is size dependent (Souli et al., 2012).

The cell membranes of the microorganisms interact with the medium so metal oxide NPs have some interactions to release metal ions that interfere with the processes of the deoxyribonucleic acid (DNA) replication, cell membrane formation and cell division (Rai *et* al., 2012).
Several studies propose that nanoparticles attach to the surface of cell membrane disturbing the permeability and respiration function of the cell. The damage to the cell may be caused by the interaction of nanoparticles with sulfur or phosphorus containing bio molecules in the cell such as DNA.

Therefore sulfur containing proteins in the membrane or inside cells and phosphorus containing Molecule like DNA are likely to be preferential sites for binding NPs. The smaller nanoparticles have more antibacterial activity that provides more surface exposure to the bacterial membrane

(Q. Li et al., 2014). In addition these nano sized particles penetrate through cell membrane easier interacting with intracellular materials and finally resulting in cell destruction in the process of multiplication (Rezaei-Zarchi et al., 2010). Documented that the nanoparticles induce oxidative stress to bacteria and induce reactive oxygen species (ROS) thus antibacterial activity could be explained based on ROS such as hydrogen peroxide (H_2O_2) Hydroxyl radicals (OH^-) and singlet oxygen (O^-). It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an electromagnetic attraction between the microbe and treated surface (Henderson, 2006).

2.6. Antibacterial activity of Cobalt Oxide nanoparticles

Currently several antimicrobial drug are used to kill microbe or prevent the growth of microbe However with the worldwide use and abuse of the most commonly used antibacterial drugs micro organisms especially bacteria are becoming resistant to more and more antimicrobial agents For example bacteria strains known to be cause tuberculosis (TB) are found to be resistant to previous effective antibacterial treatment. *E. coli* is found to be intrinsically resistant to therapeutic level of penicillin G. The first beta lactam introduced into clinical practice because of its outer membrane barrier .*E.coli* is also resistant to several different classes of antibiotics with distinct mechanism of action (Ansari *et* al., 2011).

There are many advantages among using metal NPs as antimicrobial agents due to their stability, Robustness and self-life. Synthesis of cobalt oxide NPs for antibacterial activities has been reported by researchers from leaf of *Conocarpuserectus,* leaves of *Raphanussativus var. longipinnatus* and dried powdered fruits of piper longum. Herein is reported the biofabrication of cobalt oxide nanoparticles using leaf of *Chromolaenaodorata* and their antibacterial application Nano sized cobalt oxide NPs have shown to possess antibacterial activities and several studies reported that they can be used as bactericides for water disinfection. The antibacterial activities of Cobalt oxide NPs against *E.coli* and *staphylococcus aureus* were examined. The performance of Cobalt oxide NPs for antibacterial activities prepared by biological and chemical methods to wards microbial have not been studied well. Therefore, it is important to study comparison of biologically and chemically synthesized cobalt oxide NPs for antibacterial applications (Ravishankar *e*t al., 2011).

2.7. Antibacterial strains

Staphylococci among the pathogenic bacterial strains are the common bacteria that cause a wide Varity of infections and diseases especially hurt skin and mucus membranes. On the other hand *E.coli* enteric bacteria that are currently more implicated in many digestive tract and urogental infections with the high incidence of septicemia cases.

2.7.1. Staphylococcus aureus

Staphylococci are gram positive bacteria that are characterized by individual cocci that appear to be divided into more than one plane to form grape like cluster that are none motile and none spore forming facultative anaerobes and grow by aerobic respiration or by fermentation pathogenic *S. aureus* is a member of the genus staphylococci which is named aureus due to the golden color appearance as it grown on solid media.

2.7.2. Escherichia coli

Escherichia coli is gram negative bacteria which is known to be both natural flora of humans and more over an important pathogens that considered a significant cause of morbidity and mortality worldwide. *E.coli* strains are comparatively easy to grow under both aerobic and anaerobic conditions some of *E. coli* isolates are considered part of beneficial normal flora of the intestine but some strains appeared to acquire pathogenicity mechanisms to cause disease in human such as urinary tract infection and sepsis or meningitis (A.clements *et* al, .2012).

3. MATERIAL AND METHODS

3.1. Experimental sites

Biologically and chemical synthesized Co_3O_4 nanoparticles were characterized in different Universities XRD analysis was done at Adama Science and Technology University at Department of material engineering, FTIR was done at Addis Ababa University, Determination of antibacterial activities of synthesized cobalt oxide nanoparticle was done at Oromia regional laboratory and Biology department at Adama science and technology university, UV-Vis, SEM-EDs were done at India.

3.2. Apparatus and Equipments

The apparatus and instruments used for this research work were:- Oven, UV-Vis Diffuse reflectance spectroscopy (UV-Vis DRS, Elico SL-150 spectrophotometer), X–ray diffractometer (Shimadzu, XRD-7000S South Korea), Scanning electron microscope-energy dispersive x-ray spectroscopy (SEM- EDX), Carl Zeiss Model: Neon-40,FESEM/FIB), Fourier transfer infrared spectroscopy (FTIR Perkin Elmer's spectrophotometer) , mortar ,electronic balance, beaker, PH meter, ceramic crucible, conical flask, measuring cylinder, Whatman filter paper, magnetic stirrer, centrifuge, glass filters, beaker, test tube, thermometer, watman grinding machine, hot plate, Pipets, spatulas, Erlenmeyer flask, volumetric flask, different types of beakers and aluminum foils.

3.3. Chemicals and Reagents

Double distilled water, Ethanol (extra pure) Cobalt nitrate hexa hydrated Co $(NO_3)_2.6H_2O)$ nutrient agar and sodium hydroxide (NaOH) were purchased from Addis Ababa fine Chemical shop.

3.4. Collection of the Plant Materials

Phytolaccadodecandra belongs to the family of phytolaccaceae was collected from Oromia region Arsi zone, Digelu and Tijo woreda Keble Arabi Area around Girisa river which was about 185 km from the capital city of Ethiopia Addis Ababa after conducting the field surveys.

3.5. Extraction of the leaf (broth solution)

The collected leaves of *Phytolaccadodecandra* were thoroughly washed with distilled water to remove the unwanted impurities such as dust and other particulates followed by drying allowed under shadow dry at room temperature to remove residual moisture and grinded The dried leaves of *Phytolaccadodecandra* grinded using plant grinding machine and then were packed within a plastic bottle followed by covering the whole body of the plastic bottle with aluminum foil. Extraction of the leaves of *Phytolaccadodecandra* for the biosynthesis of Co_3O_4 NPs was done by taking 16 gram of powder of *Phytolaccadodecandra* by measuring using electronic balance and was placed in to a 500 mL Erlenmeyer flask followed by addition of 250 mL of distilled water.

The Erlenmeyer flask containing the two components was placed on to a hot plate with magnetic stirrer the two components were allowed to boil at 80℃ for about 35 minutes. Then the boiled suspension formed in the solution was allowed to cool for about 15 minutes and was filtered using Whatman number one filter paper. The final extract of the solution was collected and stored at 4℃ within a refrigerator for the biosynthesis of Co_3O_4 nanoparticles within (2:1) and (1:1) ratios. The filtrate extract was used as reducing and capping agent for the biosynthesis of Co_3O_4 nanoparticles (Muniappan *et* al., 2009).

3.6. Biosynthesis of Co_3O_4 nanoparticles

Cobalt nitrate hexa hydrate is a compound with chemical formula of Co $(NO_3)_2.6H_2O$ 98%) and leaves extract of *Phytolaccadodecandra* was used for the biosynthesis of Co_3O_4 nanoparticles. To examine the effect of proportion of cobalt nitrate hexa hydrate and *Phytolaccadodecandra* leaves extract on the size and crystallite of Co_3O_4 nanoparticles 1:1 and 2:1 ratios of the *Phytolaccadodecandra* leaves extract and precursor salt were used during the biosynthesis process. Then two different samples were prepared by taking different ratios by volume of precursor salt solution and plant extract.

2:1 ratio: 66.7 mL of 0.2 M cobalt nitrate hexa hydrate and 33.3 mL of *Ph.dodecandra* extract.

1:1 ratios: 50 mL of 0.2 M cobalt nitrate hexa hydrate and 50 mL of *Ph.dodecandra* extract.

For the biosynthesis of Co_3O_4 nanoparticles within 2:1 and 1:1 ratio approximately (67.7:33.3) and (50:50) mL was taken, respectively and this was done using 0.2 M of cobalt nitrate hexa hydrate in a separate Erlenmeyer flask. In each case the Erlenmeyer flask containing the two components was placed on to a plate with magnetic stirrer without applying of heat. In each of the different ratios a small sized magnetic stirrer was placed in to the Erlenmeyer flask containing the extract and the precursor solution and the resulting solution was allowed to stir for about 1 hours and then its pH value was measured using a pH meter and then 10 ml of 2 M sodium hydroxide solution was added for each of the individual ratio as a precipitating agent. After addition of a small drop of sodium hydroxide solution for each of the individual ratios, the resulting solution was stirred for 15 minutes to maintain uniform distribution of the added base followed by centrifugation at 10,000 rpm for 15min the formed precipitate for each of the individual ratio was washed with distilled water. The formed Co_3O_4 NPs were collected using a crucible ceramic dish and placed in to a drying oven for 5 hours at 80 0℃ and then allowed to cool. The particles were store in an air tight container (Bamisaye *et* al., 2014).

3.7. Synthesis of Cobalt Oxide NPs Using Chemical Method (Co-precipitation)

Cobalt oxide nanoparticle was synthesized without any capping agents by co-precipitation method from cobalt nitrate hexahydrate precursor in the presence sodium hydroxide. 10 ML of 2 M NaOH solutions was dropped in 200 mL of 1 M Co $(NO_3)_2.6H_2O$ solution under constant magnetic stirrer for 2 hours at room temperature. The Erlenmeyer flask containing the 200 mL of 1 M Co $(NO_3)_2.6H_2O$ solution was placed on to a plate with magnetic stirrer without applying of heat a small sized magnetic stirrer was placed in to the Erlenmeyer flask containing the precursor solution and then its pH value was measured using a pH meter and then a small drop of 2 M of sodium hydroxide solution was added the resulting solution was allowed to stir for about 2 hours. Then the stirred solution was stayed overnight at room temperature The cobalt hydroxide settles down and the excess solutions found on top was discarded very carefully the stock solution of precipitates was separated using centrifuge machine centrifugation at 10,000 rpm for 15min the black color Co_3O_4 NPs precipitate was washed with distilled water and collected using a crucible ceramic dish and placed in to a drying oven for 5 hours at 80 ^oC (Rathodetal.,2015)

3.8. Characterization of Cobalt Oxide NPs Using XRD

The crystalline structure of the synthesized Co_3O_4 nanoparticles was investigated by the X-ray diffraction using X-ray diffractometer at Adama science and technology university department of material engineering XRD spectrum was recorded from 10^{o} to 80^{o} with 2θ angles using CuKα λ =1.54056 A^{o} radiation operated at 40 kV and 30 mA. Debye Scherrer‟s equation indicated in equation 1, was used to estimate the average crystallite size of the synthesized nanoparticles. (Xu et al., 2008).

$$D = \frac{0.94\lambda}{\beta cos\theta} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where D is the crystallite size, λ = 1.54 A^{o} is the X - ray source wavelength, β is the full width at half maximum intensity of the peak position. And θ is the peak position.

3.9. SEM-EDS Analysis

From both chemically and biologically synthesized nanoparticles small amount was taken and characterized by scanning electron microscopy-energy dispersive x-ray spectroscopy. SEM-EDX was provided information about the synthesized sample including morphology, chemical composition and crystalline structure It was also provided detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or backscattered electron signal (Pookmanee,2009).

3.10. Characterization of Co_3O_4 NPs using UV-Vis Spectrophotometer

Many molecules absorb ultraviolet (UV) or visible light. The absorption of UV or visible radiation can use the excitation of outer electrons from their ground state to an excited state. The Bouguer Lambert-Beer law forms the mathematical physical basis for the light absorption measurements on gas and in solution. According to this law absorbance is directly proportional to the path length l and the concentration of the absorbing substance c and expressed as $A=\varepsilon bc$ where ε is a constant of proportionality called the absorptivity. In addition absorption strongly depends on the types of samples and the environment of the sample. For instance molecules absorb radiation of various wavelengths depending on the structural groups present within the molecules and show a number of absorption bands in the absorption spectrum. The UV Vis absorption spectra of the biosynthesized Cobalt oxide nanoparticles from the leaves of *Phytolaccadodecandra* and chemically synthesized using Co-precipitation method was confirmed by using a wavelength scan between 200 nm and 800 nm.Their characterization techniques was also gave information about bandgap energy of the biosynthesized and chemically synthesized Co_3O_4 nanoparticles.

$$E_g = \frac{hc}{\lambda} \quad \ldots\ldots (2)$$

Where E_g is energy bandgap, h is planks constant, c is speed of light and λ is the maximum wavelength of the synthesized Co_3O_4 nanoparticles (López *et* al.,2010).

3.11. Characterization of Co_3O_4 nanoparticles Using FTIR

This characterization technique was carried out on biologically and chemically synthesized cobalt nanoparticles to confirm the role of extracts used during synthesis. FTIR measurement of the samples was performed at wave number range of 4000-400 cm^{-1} Measures infrared intensity was used to determine the nature of associated functional groups and structural features of biological extracts with NPs. The green and chemically synthesized cobalt oxide nanoparticle was analyzed using Fourier Transform Infrared (FTIR) Spectrometer. Also to determine the functional group in which bimolecular present in the leaves extract (Vijayalakshmi & Rajendran, 2012).

3.12. Preparation of Inoculums for Antibacterial test

Nutrient broth 28 mg dissolved in 100 ml distilled water was prepared in 2 conical flasks and sterilized. In one conical flask clinically isolated strain of *staphylococcus aureus,* was inoculated. In the other conical flask clinically isolated strain of *Escherichia coli* was added. These bacterial cultures inoculated in nutrient broth were kept on rotary shaker for 24 hours at 100 rpm.

3.13. Disc diffusion method

Antibacterial tests was carried out at Oromia regional research laboratory and Adama science and technology university at biology department by the disc diffusion method using the suspension of bacteria spread on nutrient agar (Igwe & Ekebo, 2018). Then the swab was dipped into the broth culture of the organism. The swab was gently squeezed against the inside of the tube to remove excess fluid. The swab was used to streak agar plate or a nutrient agar plate for a lawn of growth. This was best accomplished by streaking the plate in one direction then streaking at right angles to the first streaking and finally streaking diagonally. After that the swab was used to streak the outside diameter of the agar. The inoculated plates were incubated at 37 ^{o} C for 24 hours. Antibiotic discs was placed on the surface of the agar using a dispenser that dispenses multiple discs at the correct distance apart or by obtaining individual discs and placing them on the surface of the agar using flame sterilized forceps. Then antimicrobial activity was evaluated by measuring the zone of inhibition against the test Baceria staphylococcus aureus and *Escherichia coli*) diameters of zones of inhibition of the control strain and test was measured using a ruler and calipers. (Igwe & Ekebo, 2018).

4. RESULTS AND DISCUSSION

In this study cobalt oxide nanoparticle was synthesized by green and co-precipitation methods. The synthesized nanoparticles were characterized using XRD, SEM, UV Vis and FTIR and their antibacterial activity were compared. The application of synthesized NPs was investigated using both gram positive and gram negative bacterial strains.

4.1. XRD Analysis

Figure1 shows XRD patterns of Co_3O_4NPs biosynthesized from cobalt nitrate hexahydrate precursor and *Phytolacca dodecandra* leaves extract (a) and chemically synthesized using co-precipitation method (b). Cobalt oxide nanoparticles formation was analyzed by x-ray diffraction. The peaks observed in the samples at 2θ values of 36.8620, 65.2592 , 31.2878 , 19.0192 , 19.6240 , 27.2871 and 36.9203 , 65.3879 ,59.4985 , 11.5839 , 14.9650 ,15.9873 for biologically and chemically synthesized Co_3O_4 NPs, respectively along with miller indices values of (111) ,(220) ,(311), (222), (400) and (422). Sharp diffraction peak was observed in chemically synthesized Co_3O_4 nanoparticles whereas the intensity of diffraction peak of green synthesized Co_3O_4 nanoparticles is less with slight broadening. Those results reveals that the biosynthesized NPs have possessed good crystallinity in nature and also diffraction peaks related to impurities were not observed in the XRD pattern confirming the high purity of the biosynthesized nanomaterials. The average crystalline size of the biosynthesized NPs was found to be 10.79 nm where as chemically synthesized was 11.9 nm. Thus the broadening of XRD peak of green synthesized nanoparticles observed in our study confirms the size reduction. The XRD peak of chemically synthesized Co_3O_4 nanoparticles is sharp thus indicating that their size is still larger than the green synthesized Co_3O_4 nanoparticles.

The intensity of the diffraction peak of green synthesized Co_3O_4 nanoparticles is less when compared to chemically synthesized nanoparticles. We suggest that the phytochemical present in the extract of *Phytolacca dodecandra* leaves resulting in decreased intensity in XRD peak. (Löffler *et* al., 2001).

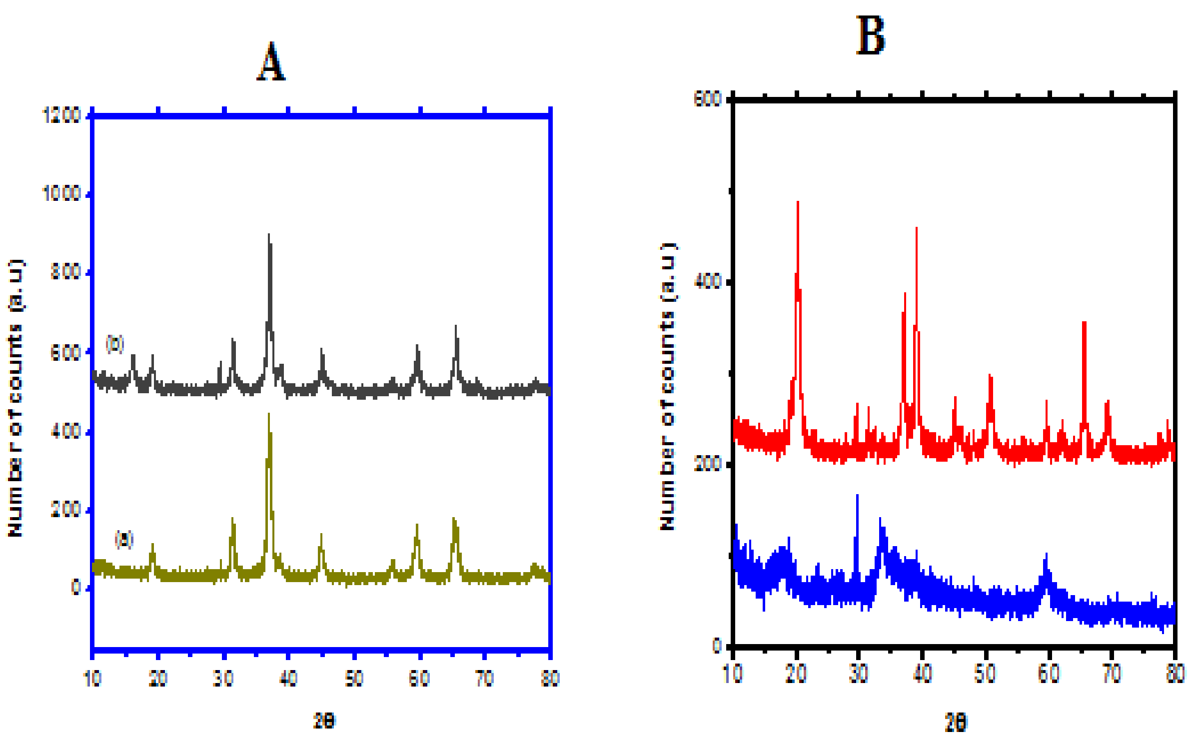

Figure 1. XRD pattern of (A) Calcinated chemically and biologically (B) Uncalcinated biologically and chemically synthesized Co_3O_4 nanoparticles.

Both calcinated Co_3O_4 nanoparticles shows sharp peak which confirms good crystallinity of the synthesized nanoparticles where as the Uncalcinated both chemically and biologically synthesized cobalt oxide nanoparticles not show sharp peak specially for the biologically synthesized one the crystallinity was absent due to excess plant extract used.

Table.1 and **2** displays structural parameters such as position (2θ), Full width at half maxima, (FWHM), miller indices (hkl) and particle size (D) for both biologically and chemically synthesized cobalt oxide nanoparticles respectively.

Table1. Structural parameters for biologically synthesized Co_3O_4 NPs .for (2:1)

S.No	**2θ (deg.)**	**FWHM**	**hkl**	**D (nm)**
1	19.2878	0.01	2,2,2	14.06
2	19.624	0.0021	4,0,0	7.13
3	27.2871	0.0063	4,2,2	21.38
4	31.2878	0.01211	3,1,1	11.89
5	36.862	0.0128	1,1,1	11.85
6	38.43	0.0128	4,0,0	24.68
7	65.2592	0.01622	2,2,0	8.62
	Average			14.23

Table 2. Structural parameters for chemically synthesized Co_3O_4 NPs.

S.No	**2θ (deg.)**	**FWHM**	**Hkl**	**D (nm)**
1	11.5839	0.23	2,2,2	13.93
2	14.965	0.3	4,0,0	8.93
3	15.9873	0.63	4,2,2	21.793
4	16.6109	0.5066	3,1,1	26.22
5	36.862	0.05797	1,1,1	12.97
6	59.4985	0.588	4,0,0	11.68
7	65.3879	0.5973	2,2,0	11.15
	Average			15.2

4.2. SEM Analysis

The morphology of the biologically and chemically synthesized Co_3O_4 nanoparticles was viewed through the high resolution field emission scanning electron microscopy (SEM). Figure 2(a) and (b) shows the SEM images of the biologically and chemically synthesized cobalt oxide nanoparticles, respectively. The SEM image reveals the surface morphology of Co_3O_4 nanoparticles and the particles were found to be spherical shape. The image also elucidates the decrease of particles size with the biologically synthesized using leaves extract of *Phytolacca dodecandra* implies the well association of biomolecules obtained from the leaves extracts with that of Co_3O_4 precursor salt during the biosynthesis process and the presence of the leaves extract coats the surface of the Co_3O_4 NPs thus preventing their aggregation.

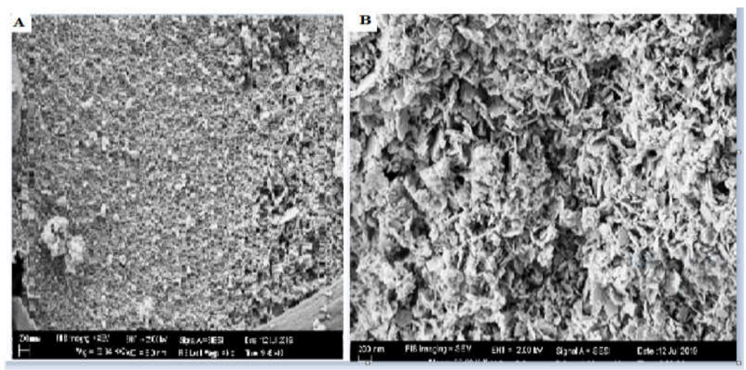

Figure 2. Scanning electron micrograph (SEM) image of (A) biologically and chemically (B) synthesized Co_3O_4 NPs.

4.2.1. Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

The energy dispersive X-ray analysis of biologically and chemically synthesized Co_3O_4 NPs were shown in Figures 3. It is evident from the X-ray patterns of all the samples in which Co and O are found in the respective spectra. The energy dispersive X -ray spectroscopy (EDX, Figure 3 (a and b) analysis showed the strong peaks at 0.5, 0.7, 6.8 and 7.5 keV and composition of Co and O atom in chemically synthesized Co_3O_4 NPs was larger than biologically synthesized this is due to the much amount precursor used in chemically synthesized.EDX also shows the biologically synthesized Co_3O_4 NP has a good crystallinity compared to chemically synthesized.

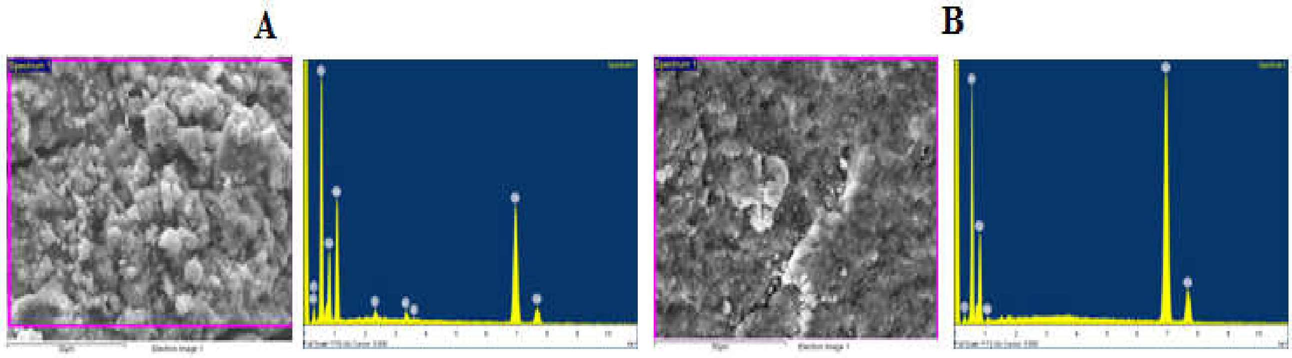

Figure 3. Energy dispersive X-ray spectra and selected area of SEM images (A) biologically and chemically (B) synthesized Co_3O_4 NPs.

Table 3: Shows quantitative analysis of biologically and chemically synthesized nanoparticles

Biological(A)			Chemical (B)		
Element	Weight %	Atomic%	element	Weight %	Atomic%
Ck	7.85	14.77	Ck	4.15	10.28
Ok	37.76	53.37	Ok	29.86	55.60
Nak	17.82	17.53	Nak	0.97	1.25
Kk	0.60	0.35	Cok	65.03	32.87
Cok	35.46	13.61			

4.3. Ultraviolet-Visible Spectroscopy (UV-Vis DRS) Analysis

The bandgap energy of synthesized nanoparticles was estimated by using ultra violet visible diffuse reflectance spectroscopy (UV –Vis DRS). The band gap is the minimum energy needed for an electron to be excited from the top of the valence band to the bottom the conduction band once that minimum energy is reached (the bandgap energy) then the sample can start absorbing light and electrons are excited from the valence band to the conduction band.

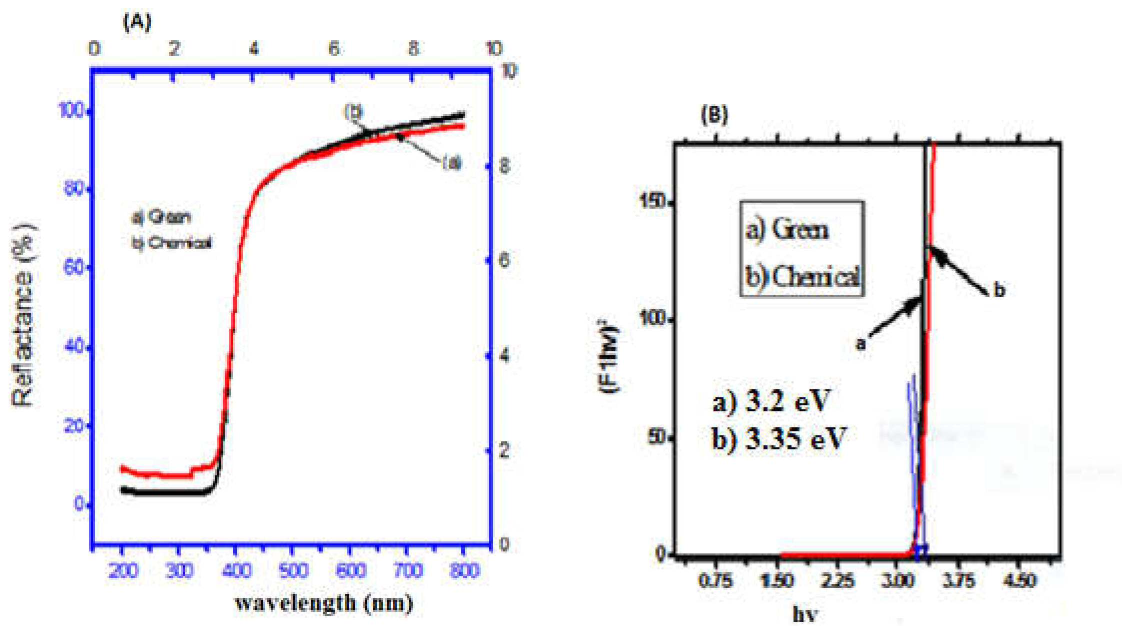

Figure 4. (A) Reflectance and (B) energy bandgap of both Green (a) and chemically (b) synthesized Co_3O_4 NPs.

Energy bandgap was plotted by using Kubelka munk function from UV-Vis diffused Reflectance spectroscopy (DRS) as described from figure 6. Bandgap energy and crystalline size of nanoparticles have inverse relationship. Cobalt oxide nanoparticles synthesized by chemical method poses high bandgap energy as compare to green synthesized Co_3O_4 NPs, this is due to the fact that small size nanoparticles had high surface area to volume ratio that helps to reflect the incident light. Therefore the bandgap energy of Co_3O_4 NPs is found to be 3.2 eV and 3.35 eV for biologically and chemically synthesized Co_3O_4 NPs, respectively. If the size of the particle is very small light interacts with the samples instead of absorption with parts of the light scattered and reflected (Rajabia A *et* al,. 2016).

4.4. Fourier Transform Infrared (FTIR) Analysis

The functional groups that could cap the surface of the particles were confirmed by FTIR studies. Figure 3 Shows the FTIR spectrum of (A) green synthesized Co_3O_4 nanoparticles from leaves extract of *Phytolacca dodecandra* (B) chemically synthesized Co_3O_4 NPs and (C) Calcinated biosynthesized Co_3O_4 nanoparticles a broad band was observed between 4000 to 400 cm^{-1} spectra of the cobalt oxide nanoparticles synthesized from *Phytolacca dodecandra* leaf extract and chemically synthesized the spectrum of cobalt oxide nanoparticles corresponding to O-H stretch-H stretch, C=C stretch C-H stretch, and C-O-C stretch.

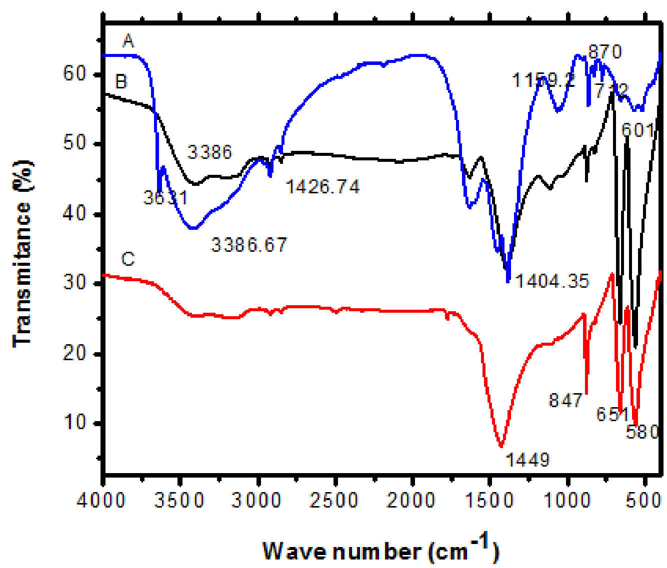

Figure 5. (A) Uncalcinated (B) calcinated and chemically synthesized (C) Co_3O_4 nanoparticles.

The peaks are suggesting at 870 and 712 cm^{-1} indicates the presence of C-H out-of plane bending of aromatics. Similar observations on the association of nanoparticles with other molecules have been reported by other researchers. The broad absorption peak located at 1159.2cm^{-1} confirm water-soluble phytochemicals present in the leaf extract of *Phytolacca dodecandra*, that have the ability to perform dual functions of reduction and stabilization of the Co_3O_4 NPs (Caroling G., *et* al.2015). Figure 3(C) shows FTIR spectra of chemically synthesized Co_3O_4 through co-precipitation method. The strong bands at 580 and 847 cm^{-1} belonging to the spinel structure of the Co_3O_4 NPs. The broad peak located in between 847-651 cm^{-1} is attributed to the stretching vibration mode of Co-O in which Co is Co^{2+} and is tetrahedral coordinated. The later one at 580

cm^{-1} can be assigned to Co-O of octahedral coordinated Co^{3+}. The two bands appeared at 3631 and 1406 cm^{-1} have been assigned to the stretching and binding vibrations of absorbed water molecule with Co_3O_4 NPs (Teng,.*et* al.2010).

4.5. Antibacterial studies

Co_3O_4 nanoparticles at concentrations of 25µg/ml and 50µg/ml were tested for antibacterial activity using gram-negative and gram-positive bacteria strains of *Escherichia coli* and *Staphylococcus aureus* for both biologically and chemically synthesized Co_3O_4 nanoparticles using disc diffusion method. The antibacterial activity of Co_3O_4 nanoparticles was compared with the positive control ciprofloxacin; the results of antibacterial studies clearly suggest that Co_3O_4 nanoparticles synthesized using *Phytolacca dodecandra* leaves extract Table 4 and 5 shows even at 25µg/ml has better antibacterial activity. when compared to chemically synthesized Co_3O_4 nanoparticles. These human pathogen bacteria strains are capable of causing diseases ranging from skin infections, meningitis, bacteraemia, urinary tract infections, vomiting, diarrhea, anemia, kidney infections. The surfaces of the cobalt oxide nanoparticles might have interacted directly with the bacterial outer membrane causing the membrane to rupture and killing them. So, the antibacterial activity exhibited by the chemically and biologically synthesized cobalt oxide nanoparticles is attributed to their small size and high surface area to volume ratio which allows them to interact closely with microbial cell membrane.

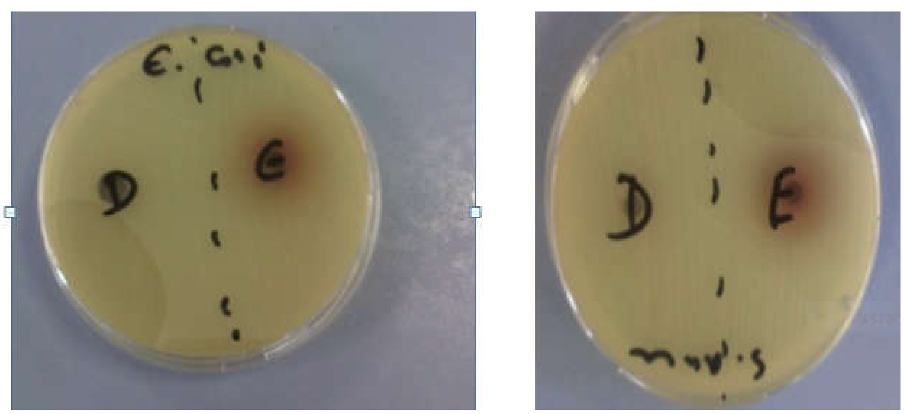

Figure 6. Antibacterial activity of biologically (E) and chemically (D) synthesized Co_3O_4 Nps 25µg/ml.

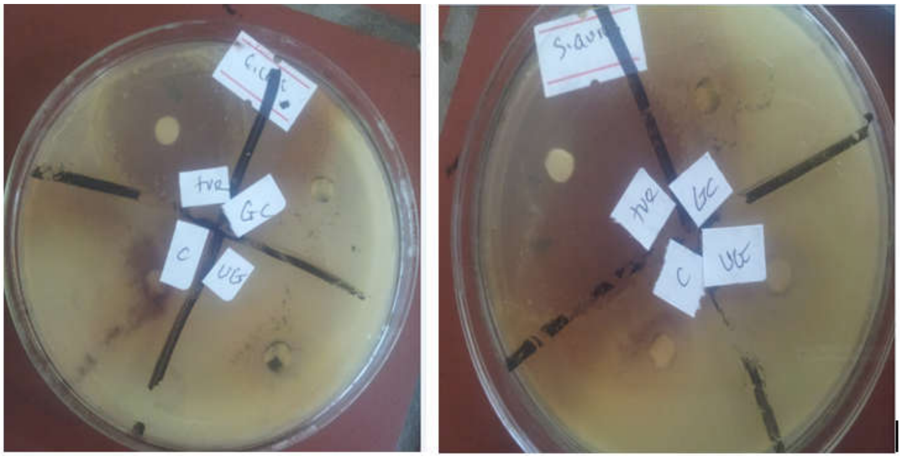

Figure 7. Antibacterial activity of biologically (GC.2:1),(UG.1:1) and chemically (C) synthesized Co_3O_4 Nps 50µg/ml Positive control ciprofloxacin.

Table 4:. Zone of inhibition (mm) of biologically (E) and chemically (D) synthesized Co_3O_4 Nps 25µg/ml.

S.No	**Bacteria**	**concentration**	**+ve control**	**Green**	**Chemical**
		25µg/ml	ciprofloxacin	(E)	(D)
1	*E.coli*		14.5	4.5	2.8
2	*S.aureus*		14	2.3	2.11

Table 5: Zone of inhibition (mm) of biologically (GC.2:1), (UG.1:1) and chemically (C) synthesized Co_3O_4 Nps 50 µg/ml.

S.No	**Bacteria**	**concentration**	**+ve control**	**Green**		**Chemical**
		50 µg/ml	ciprofloxacin	GC	UG	C
1	*E.coli*		27.3	12.5	11	11
2	*S.aureus*		25.5	11.6	12.5	12

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In this study we have synthesized Co_3O_4 nanoparticles using *Phytolacca dodecandra* leaves extract as capping agent and chemical methods using co-precipitation. Both nanoparticles were characterized for their size and crystallinity using XRD, capping photochemical by FTIR, morphology by SEM and Energy bandgap by UV-vis The Co_3O_4 nanoparticles which was capped and stabilized leaves extract was found to be lesser in size and more dispersed than the Co_3O_4 nanoparticles prepared by chemical method. More over the *Phytolacca dodecandra* extract stabilized Co_3O_4 nanoparticles exhibited considerable antimicrobial activity against pathogenic bacteria which is comparable with that of standard antibiotic. Based on these results we conclude that the leave extract stabilized Co_3O_4 nanoparticles have potential biomedical applications when compared to chemically synthesized Co_3O_4 nanoparticles due to its enhanced dispersibility, stability and small size.

5.2. Recommendations

Based on the present findings, cobalt oxide nanoparticles could be synthesized by green method as a result to write chemical reaction mechanisms between precursor salt and extract, structural characterization of the extract should be done using NMR and as a result it could be possible to estimate antibacterial mechanism with cobalt oxide nanoparticles.

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