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RESEARCH PAPER

Synthesis of Cobalt Oxide Nanoparticles Through Chemical and Biological Pathways for Antibacterial Activity

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ABSTRACT

Green synthesis of nanoparticles (NPs) using different parts of plant extracts is a novel and environmentally benign method that can be used in numerous biomedical applications. In this study, cobalt oxide nanoparticles were synthesized through biological method using 0.2 M cobalt nitrate hexahydrate as a precursor salt and leaf extract of indigenous plant of Ethiopia called Phytolacca dodecandra as a reducing and capping agent. In addition to this, cobalt oxide nanoparticles were synthesized chemically by co-precipitation method from cobalt nitrate hexahydrate in the presence of sodium hydroxide as a precipitating agent. The synthesized Co_3O_4 NPs were characterized using X-Ray diffraction (XRD), scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDX), Ultraviolet-Diffuse Reflectance spectroscopy (UV-Vis-DRS) and Fourier transform infrared (FT-IR) spectroscopy. The average crystal size Co_3O NPs were found to be 10.79 nm and 11.9 nm, having band gap energy of 3.35 eV and 3.18 eV for the biologically and chemically synthesized Co_3O_4 NPs, respectively. The shape and morphology of Co_3O_4 NPs synthesized in the two methods were found to be spherical. XRD and FT-IR analyses have confirmed the formation of Co_3O_4 NPs from its precursor salt in the presence of Phytolacca dodecandra leaf extract. The antibacterial activity of both the calcined and un-calcined biologically and chemically synthesized Co_3O_4 NPs were found in the range of 8.3-12.5 mm. As compared to the chemically synthesized Co_3O_4 NPs, biologically synthesized Co_3O_4 NPs shows high antibacterial activity due to the production of high reduced reactive oxygen species (ROS) because of the presence of Phytolacca dodecandra leaf extract and due to the relatively small average crystalline size as compared to chemically synthesized Co_3O_4 NPs.

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INTRODUCTION

Nowadays, the industries of water treatment, cosmetics, medicine, energy and pharmaceutics have been using nanoparticles largely for their suitability and multi-functionalities [1]. Due to the widespread applications of those different types of nanoparticles in different technologies and biotechnological aspects; currently the synthesis of different kinds of nanoparticles via different protocols became very essential [2]. Nanoparticles

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with various compositions and applications can be prepared by physical, chemical and biological methods [3]. The physical synthesis methods include evaporation, sputtering, laser ablation, ion ejection and electron- beam lithography [4]. The chemical synthesis methods include salt reduction, reverse micelles, electrochemical, sol-gel, gas-liquid interface, thermolysis, co-precipitation and decomposition on ultrasonic treatment [5]. However, such mentioned methods involve the use of expensive and toxic chemicals as a capping agent, reducing agent, and as solvent during the synthesis process. Those synthesis protocols by themselves require high temperature, expensive and complicated instruments; and the method also involves the releasing of by products that affect the natural environment. Instead, green synthesis method is an environment-friendly approach to synthesize different types of nanoparticles for various applications. This method involves the use of biological available and cheap resources in nature such as extracts of green plants, algae, fungi, yeast, bacteria and viruses for the formation of stable nanoparticles for various novel applications [3].

Cobalt oxide is a transition metal oxide and has special characteristics such as high surface area: due to their small size; high magnetic behavior, 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 [6]. Cobalt Oxide nanoparticles have been using in wide range of applications from energy through water treatment to biomedical and as antimicrobial agent [6].

Nowadays, one of the global health concerns is the increasing antimicrobial resistance of many microorganisms against drugs, because some pathogens which were curable in past now are becoming untreatable such as Methicillin-resistant staphylococcus aureus (MRSA) [7]. To win this battle and to overcome this frightening situation of microbial resistance to antibiotics, using metal oxide nanoparticles synthesized via easily achievable cost with easy techniques are becoming as the best alternative options worldwide [8]. Cobalt oxide nanoparticles are one of the inorganic nano materials which can be used for such antimicrobial activities because of its interesting various physio-chemical properties [9]. Towards this end, cobalt oxide nanoparticles were synthesized via green method in the presence of the leaf extract of Phytolacca dodecandra as capping and reducing agent and chemical (coprecipitation) method; and then the nanoparticles synthesized in these two methods were evaluated against selected drug resistance human pathogen bacteria species of Staphylococcus aureus and Escherichia coli followed by comparing their antimicrobial potential applications.

Of course, synthesis of cobalt oxide nanoparticles through different methods for different potential applications has been already reported previously [10]. However, up to the knowledge of the authors of this study, synthesis of nanoparticles using the leaf extract of Phytolacca dodecandra as capping and reducing agent for antibacterial applications has not been reported before. In addition to this, cobalt oxide nanoparticles never synthesized via co-precipitation method and a comparative study of antibacterial never done yet. Therefore, the present study aims to synthesize cobalt oxide NPs in co-precipitation and green method using leaf extract of Phytolacca dodecandra and studying its application for antibacterial activity; comparing the efficiency of the synthesized cobalt oxide NPs prepared using these two methods on both gram negative and gram-positive bacteria strains.

MATERIALS AND METHODS

Chemicals

Chemicals, reagents, and solvents used during this work includes distilled water, absolute ethanol (99.9%, LabTech Chemicals), ethanol reagent (97%, LabTech Chemicals), Cobalt nitrate hexahydrate (98%, Sigma Aldrich), acetone (Sigma Aldrich), Dimethyl sulfoxide (DEMSO, Sigma Aldrich) and Müller-Hinton agar (Sigma Aldrich). All these chemicals and reagents are of analytical grades and as a result were used without performing any further purification. Instruments Used

Different instruments such as FT-IR spectrophotometer (PerkinElmer 65), XRD (XRD-7000, Shimadzu Co., South Korea), UV-Vis spectrophotometer (JASCO V-78 UV-Vis spectrophotometer equipped with a diffuse reflectance attachment for powder samples), field emission scanning electron microscopy-SEM-EDX (FESEM, JEOL-JSM 804081F, Japan) were used for characterizing of functional groups, crystallinity nature, optical property, surface morphology and elemental analysis of green and chemically

synthesized Co_3O_4 NPs, respectively.

Leaf Collection and Extraction (Broth Solution Preparation)

The leaves of Phytolacca dodecandra were collected from Digelu and Tijo district in Arsi Zone, Oromia Regional State, Ethiopia. The collected leaves of Phytolacca dodecandra were thoroughly washed with distilled water to remove the unwanted impurities such as dust and other particulates followed by drying under shaded at room temperature to remove all residual moisture content. The dried leaves of Phytolacca dodecandra were grinded using plant grinding machine followed by packing the powder within a plastic bottle. Extraction of the leaves was done by mixing 16 grams of powder of Phytolacca dodecandra and 250 mL of distilled water in a 500 mL Erlenmeyer flask.

The Erlenmeyer flask containing the mixture was placed on to a hot plate with magnetic stirrer and boiled at 80℃ for about 35 minutes. Then the boiled suspension was allowed to cool for about 35 minutes and was filtered using Whatman number one filter paper. The extracted solution of Phytolacca dodecandra leaves was stored in a refrigerator at 4℃ for the biosynthesis of Co_3O_4 nanoparticles [11]. Fig.1 shows picture of Phytolacca dodecandra plant.

Biosynthesis of Co_3O_4 Nanoparticles

The solutions of leave extracts of Phytolacca dodecandra prepared in section 2.3 and that of cobalt nitrate hexahydrate [Co $(NO_3)_2.6H_2O$], were used for synthesis of Co_3O_4 nanoparticles. So, to compare the antibacterial activities of Co_3O_4 nanoparticles prepared in the two methods, synthesis of $Co_3O_{4\ NPs}$ was done through chemical and green method in the presence of the leaf extract of Phytolacca dodecandra). For synthesis, 0.2 M solutions of Co $(NO_3)_2.6H_2O$ was mixed with the leave extract of Phytolacca dodecandra. The resulting solution of Phytolacca dodecandra and Co $(NO_3)_2.6H_2O$ were left under stirring for about 1 hour and then the pH values of the solution were monitored using a pH meter. Then to the solution mixture, 2.0 M sodium hydroxide solution was added in drowse manner to facilitate the formation of precipitation. The resulting suspension in the solution flask was precipitated with help of centrifugation at 1000 rpm for 15 minutes and followed by washing with distilled water and absolute ethanol sequentially. The formed Co_3O_4 NPs were collected using a crucible dish and then were dried using drying oven for 5 hours at 80. The particles were store in an air tight container for further analysis and characterization [12].

Synthesis of Co_3O_4NPs Using Co-precipitation Method

Cobalt oxide nanoparticles were synthesized without the addition of any capping/stabilizing agents through co-precipitation method using Co $(NO_3)_2.6H_2O$ in the presence sodium hydroxide as a precipitating agent. For this procedure 2.0 M of NaOH was taken and added step wise into a solution of 200 mL of 1.0 M Co $(NO_3)_2.6H_2O$ precursor under constant magnetic stirring for about 2 hours at room temperature. Then the pH value of the resulting suspension was monitored using a pH meter. The suspension was left overnight at room temperature. Then after the formed precipitate of hydroxide was settled down with the excess and clear solution found on top. The clear solution was discarded very carefully from the formed precipitate. The formed precipitate was then separated from the rest of solution by centrifugation at 1000 rpm for 15 minutes three times separately. The black color Co_3O_4 precipitate was washed with distilled water followed by absolute ethanol repeatedly and then the precipitate was collected using crucible ceramic dish, and dried in oven at temperature at 80 for about 5 hours [13].

Characterization of Synthesized Co_3O_4NPs

The crystalline structure of the synthesized Co_3O_4 nanoparticles was analyzed using X-ray diffraction measured by X-ray diffractometer (XRD-7000, Shimadzu Co., South Korea). The average crystalline size of the synthesized Co_3O_4 nanoparticles were calculated from XRD data. The morphology and shape of the synthesized cobalt oxide NPs was confirmed using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDX) (Carl Zeiss Model: Neon-40, FESEM/FIB, SMT, AG, Germany). Optical band gap of the synthesized Co_3O_4 NPs were characterized using UV-Vis-Diffuse Reflectance Spectrophotometer (Elico SL-150 spectrophotometer, UV-Vis spectrophotometer equipped with a diffuse reflectance attachment for powder samples). Information about the

functional groups was obtained by characterizing both green and chemically synthesized NPs using FTIR techniques (Perkin Elmer65, PerkinElmer, Inc., Waltham, USA).

Antibacterial Activity Studies
Preparation of Inoculum

First 2.5 mg nutrient broth was taken followed by dissolving it within 100 mL of distilled water and then was prepared within two 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 were inoculated in nutrient broth and were kept on rotary shaker for about 24 hours at 100 rpm [14].

Disc diffusion method

Antibacterial tests were carried out by disc diffusion method using the suspension of bacteria spread on nutrient agar followed the modified method [15]. Then the swab was dipped into the broth culture of the bacteria. 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 for 24 hours. Antibiotic discs were 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 the antimicrobial activity was evaluated by measuring the diameter of zone of inhibition against the tested bacteria of Staphylococcus aureus and Escherichia coli and was measured using a ruler and calipers [15].

RESULTS AND DISCUSSION

X-Ray Diffraction (XRD) Analysis

Fig.2 shows the XRD pattern of Co_3O_4 NPs biosynthesized from cobalt nitrate hexahydrate precursor salt and Phytolacca dodecandra leaf extracts (through biological method) and chemically synthesized Co_3O_4 NPs obtaining (co-precipitation method). Effects of calcination on Co_3O_4 NPs were manifested by XRD patters between Fig.2 A and Fig.2 B. The sharp and intense peaks of XRD in Fig.2A demonstrated that calcined samples were highly crystalline for both Co_3O_4 NPs synthesized in green and chemical methods. The XRD patters labeled by (a) and b) in Fig.2B

Fig. 1. Photo of the plant Phytolacca dodecandra.

shows that Co_3O_4 NPs were less crystalline (both for chemically and green methods of synthesis) in uncalcined samples.

The peaks observed at 2θ values of 19, 31.22, 37, 44.9, 59.22 and 65.18 for biologically for and 18.90, 31.26, 36.78, 45.02, 59.50, and 65.26 for chemically synthesized Co_3O_4 NPs, respectively along with miller indices values of (111), (220), (311), (222), (400) and (422). The average crystalline size of biologically and chemically synthesized Co_3O_4 NPs were found to be 10.79 nm and 11.9 nm, respectively. Sharp diffraction peaks were observed in chemically synthesized Co_3O_4 nanoparticles whereas the intensity of diffraction peaks of biologically synthesized Co_3O_4 nanoparticles is less with slight broadening. Those results reveal that biosynthesized Co_3O_4 NPs have possessed good crystallinity in nature and also diffraction peaks related to impurities were not observed in the green synthesized XRD pattern of Co_3O_4 NPs; confirming the high purity of the biosynthesized nanomaterials. Thus, broadening of XRD peak of biologically synthesized Co_3O_4 NPs observed in the present study confirms the size reduction; due to the presence of Phytolacca dodecandra leaf extract that plays great role as both capping and reducing agent and thus prevents Co_3O_4 NPs over growth formation. As it can be observed, the XRD peak of chemically synthesized Co_3O_4 nanoparticles is sharp, this indicating that their size is still larger than the biologically synthesized Co_3O_4 nanoparticles. As it can be revealed under the XRD spectra, chemically synthesized Co_3O_4 NPs are free of impurities as no additional and foreign peaks are not observed like that of green mediated synthesized Co_3O_4 NPs. As compared to the green calcined Co_3O_4 NPs, uncalcined green synthesized Co_3O_4 NPs shows vibrations due to the existence of some bioactive molecules from the leaf extract of Phytolacca dodecandra.

SEM-EDX Analysis

The morphology and the chemical composition of both biologically and chemically synthesized Co_3O_4 nanoparticles were viewed through the high-resolution field emission scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDX). Fig. 3(a) and (b) shows the SEM images of biologically and chemically synthesized cobalt oxide nanoparticles, respectively. The SEM image (Fig.3) and SEM-EDS (Fig.4) reveal that shape and morphology of Co_3O_4 nanoparticles were found to be spherical in shape. The image also elucidates the decrease of particle size for the biologically synthesized Co_3O_4 nanoparticles as compared to the chemically synthesized Co_3O_4 NPs ; implies the well association of biomolecules obtained from the leave extract with that of Co_3O_4 precursor salt during the biosynthesis process and the presence of the leaves extract coats the surface of Co_3O_4 NPs, thus preventing from aggregation and agglomeration and so results in a relatively homogenized morphonology.

The energy dispersive X-ray analysis of biologically and chemically synthesized Co_3O_4 NPs was shown in Fig. 4. It is evident from the X-ray patterns of all the samples in which Co and O are

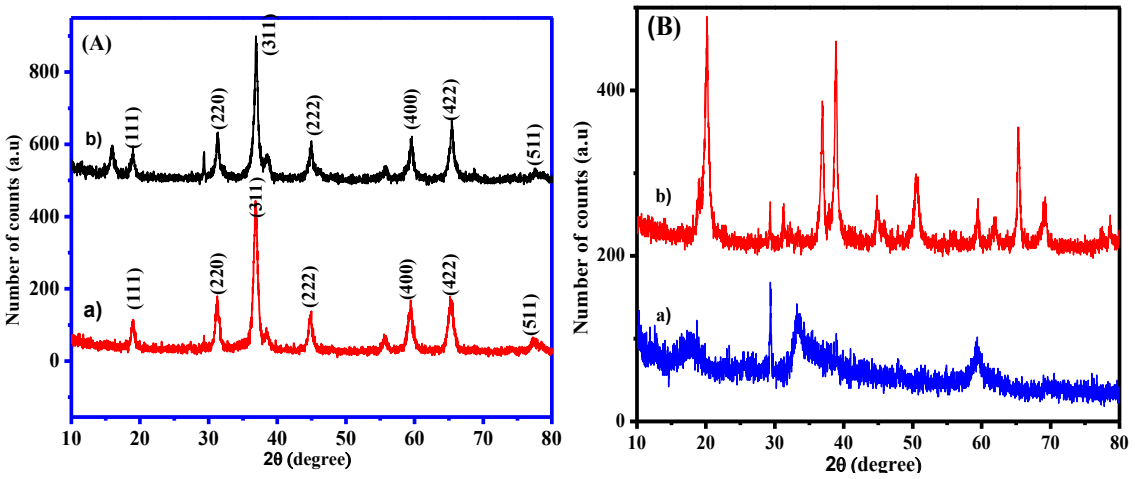

Fig. 2. XRD pattern of (A) calcined Co_3O_4 NPs synthesized in a) chemical and b) green method; and (B), XRD pattern of uncalcined Co_3O_4 NPs synthesized in a) green and b) chemically method.

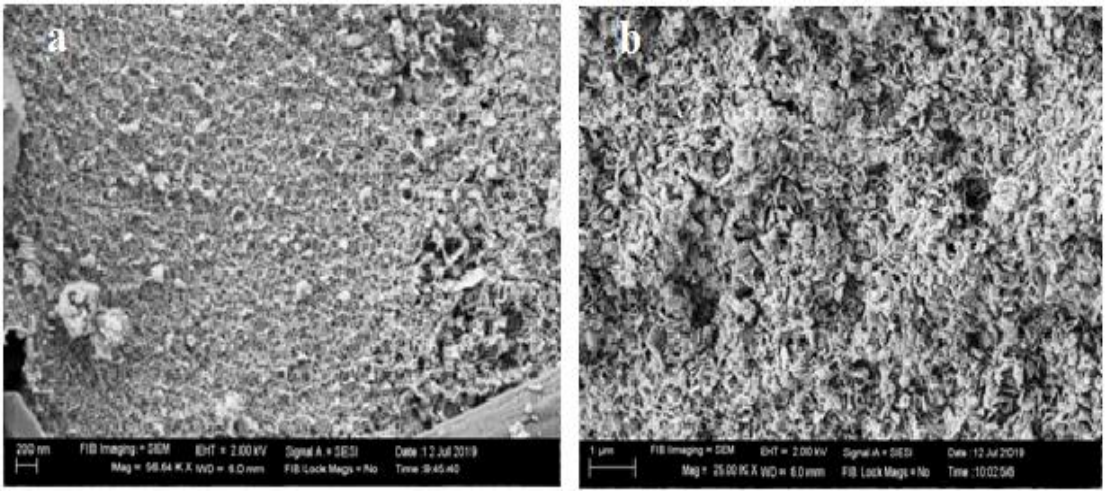

Fig. 3. SEM images of (a) biologically and (b) chemically synthesized Co_3O_4 NPs.

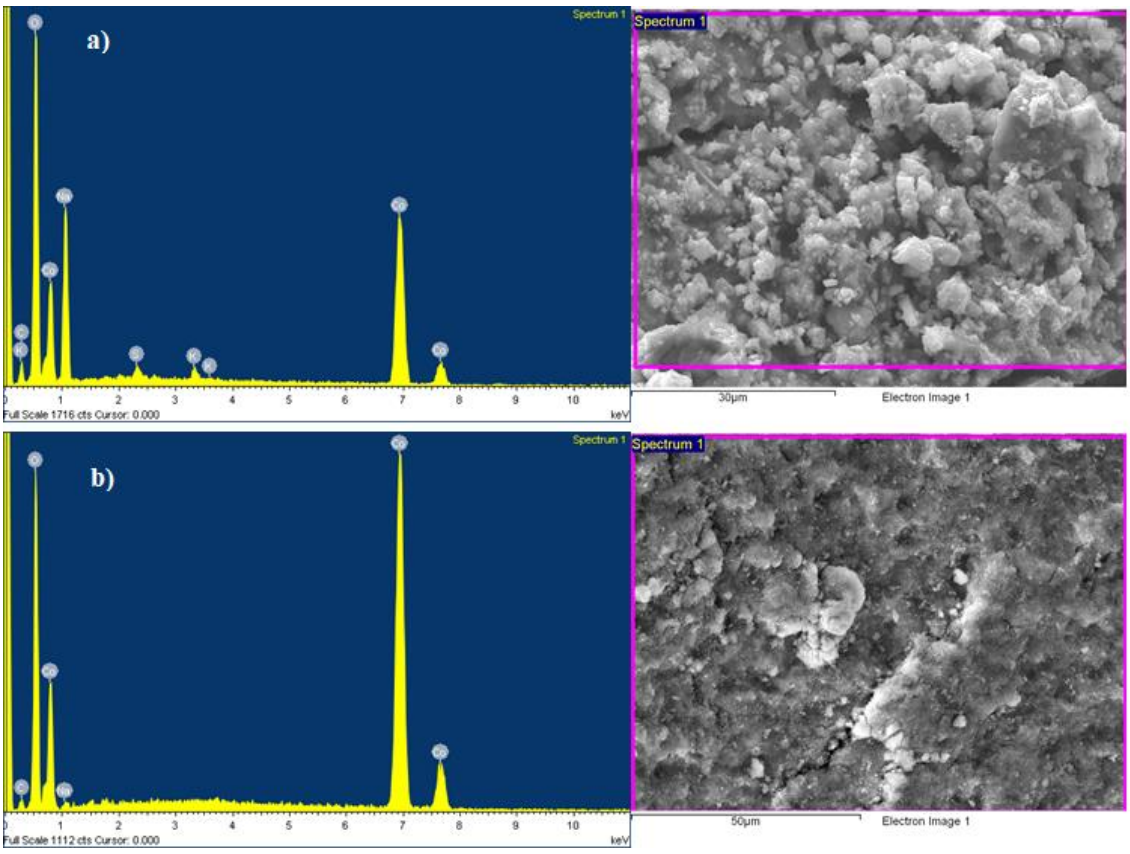

Fig. 4. SEM-EDS spectra of (a) biologically and (b) chemically synthesized Co_3O_4 NPs.

found in the respective spectra. In addition to this, energy dispersive X-ray spectroscopy analysis showed the composition of Co_3O_4 NPs contains only the element Co and O atom; this reveals that the synthesized Co_3O_4 NPs obtained using the two methods are free from any impurities and foreign materials. Some extra elements such as C, S, and K are observed under the EDX images of Co_3O_4 NPs, which could be comes from ceramic materials during the preparation of paste for SEM-EDX

characterization, and also the existence of C like materials will be obtained from the decomposition and reaction of atmospheric carbon dioxide and in another way it might be from few remnants of leaf extract of Phytolacca dodecandra used during synthesis process as supported by related previously reported works [16, 17]. EDX also shows that biologically synthesized Co_3O_4 NPs has a good crystallinity nature as compared to chemically synthesized Co_3O_4 NPs. The absence of any foreign material other than the required synthesized Co_3O_4 NPs indicates that the synthesized Co_3O_4 NPs are highly pure.

Ultraviolet-Visible Diffuse Reflectance Spectroscopy (UV-Vis DRS) Analysis

The optical behavior of the synthesized Co_3O_4 nanoparticles was investigated by using ultra violet visible diffuse reflectance spectroscopy (UV-Vis DRS). Fig. 5 displays the Tauc plots of both green and chemically synthesized cobalt oxide nanoparticles extracted from the corresponding UV-Vis DRS datas. Energy bandgaps of Co_3O_4 NPs synthesized in these two different methods were determined from Tauc plot using Kubelka-Munk functions. The bandgap energies of Co_3O_4 NPs were found to be 3.35 eV and 3.18 eV, respectively for the biologically and chemically synthesized Co_3O_4 NPs.

Cobalt oxide nanoparticles synthesized by chemical method possess high band gap energy as compared to green synthesized Co_3O_4 NPs this is due to quantum confinement difference between nanoparticles synthesized in biological and chemical precipitation methods. Higher bandgap nanoparticles (synthesized in chemical method) possess high quantum confinement than lower bandgap-nanoparticles (synthesized in biological method). Therefore, as it can be studied in the present work, if the average size of the particles is very small, light interacts with the samples instead of absorption with parts of the light scattered and reflected back [17, 18].

Fourier Transform Infrared (FT-IR) Analysis

FTIR spectral analysis of both the biosynthesized and chemically synthesized Co_3O_4 NPs are shown in Fig. 6. The functional groups of the molecules in the plant extract that are responsible for capping and stabilizing of nanoparticles were confirmed by major bands at 3636, 3423, 1634 and 1055 cm-1 in the FT-IR spectrum. The two intense bands appeared at 3635 and 1055 cm^{-1} have been assigned to the stretching and binding vibrations of hydroxyl group of phenolic compound found in uncalcined Co_3O_4 NPs that is emanated from the

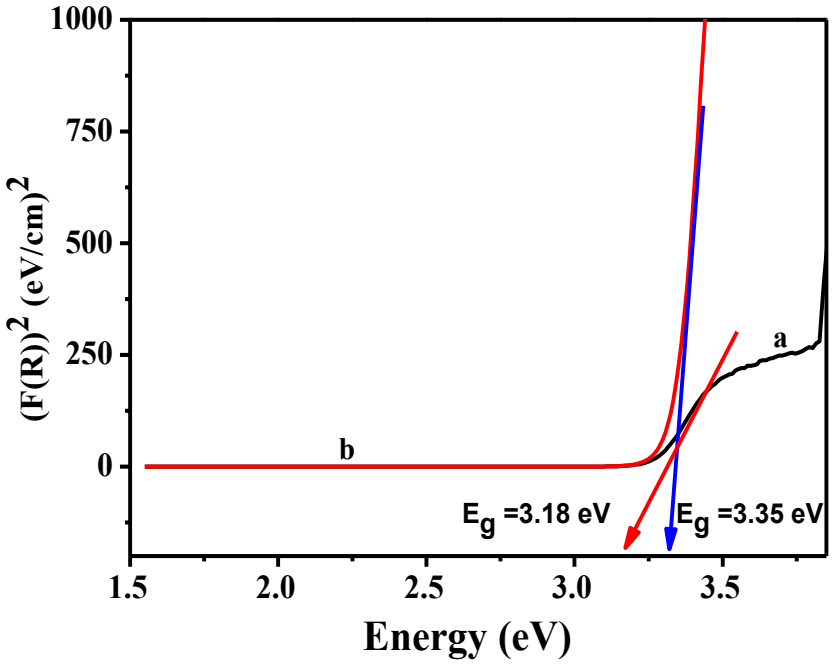

Fig. 5. Tauc plots of a) chemically and b) green (biologically) synthesized Co_3O_4 NPs and their calculated bandgaps.

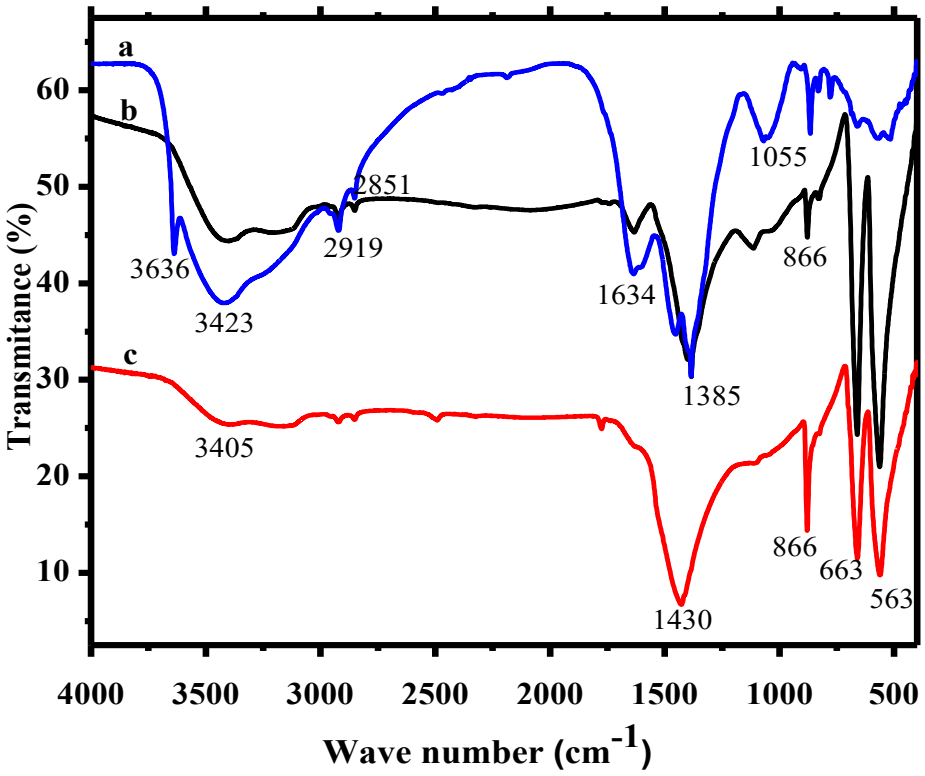

Fig. 6. (a) Uncalcinated, (b) calcinated and (c) chemically synthesized FT-IR spectra of Co_3O_4 nanoparticles.

plant extract (Fig. 6a); and broad bands appeared at 3405 cm^{-1} was assigned to O-H stretching vibration mode emanated from moistures adsorbed on the surface of calcined Co_3O_4 NPs. The major bands at 1634 cm^{-1}, 1055 cm^{-1} and 563 cm^{-1} (in curve a) can be attributed to the carbonyl functional groups, amides and Co_3O_4 NPs respectively in the uncalcined sample [19, 20].

The broad and intense absorption band located at 1385 cm^{-1} (in Fig. 6a and 6b) and at1430 cm^{-1} (in Fig.6c) confirms the presence of carbonyl functional groups emanated from the leaf extract of Phytolacca dodecandra and CO_2 gas molecules adsorbed on Co_3O_4 NPs respectively [15, 19]. Fig.6 (a), Fig. 6(b) and Fig. 6(c) shows the FT-IR spectra of uncalcined biosynthesized, calcined biosynthesized and chemically synthesized Co_3O_4NPs respectively. The strong bands at 563 and 663 cm^{-1} belonging to cobalt-oxygen bond stretching vibration that confirms formation of Co_3O_4 nanostructure [21-24]. Disappearance of bands at 3636 cm^{-1}, 29192851 cm^{-1}, 1634 cm^{-1} and 1055 cm^{-1} in the case of calcined Co_3O_4 NPs (Fig.6b) and in chemically synthesized Co_3O_4 NPs confirms removal of organic compounds after calcination [15].

Antibacterial studies of Co_3O_4 NPs

The antibacterial activity of the synthesized Co_3O_4 nanoparticles was investigated against both gram negative and gram- positive bacteria strains using disc diffusion method. To analyze the effect of nanoparticles concentration on antibacterial activity, 25µg/mL and 50µg/mL of biologically and chemically synthesized Co_3O_4 nanoparticles were prepared and tested against Escherichia coli and Staphylococcus aureus bacteria strains. The antibacterial activity of Co_3O_4 nanoparticles was compared with the positive control ciprofloxacin; the results of antibacterial studies clearly suggests that, Co_3O_4 nanoparticles synthesized through biological method shows better antibacterial activity as compared to chemically synthesized Co_3O_4 nanoparticles, even at low concentration with concentration of 25µg/mL. This is due to the fact that, in biologically synthesized Co_3O_4 nanoparticles, the production of reactive oxygen species (ROS) is high to inhibit or kill the bacteria due to the presence phytochemicals emanated from Phytolacca dodecandra [25]; and also due

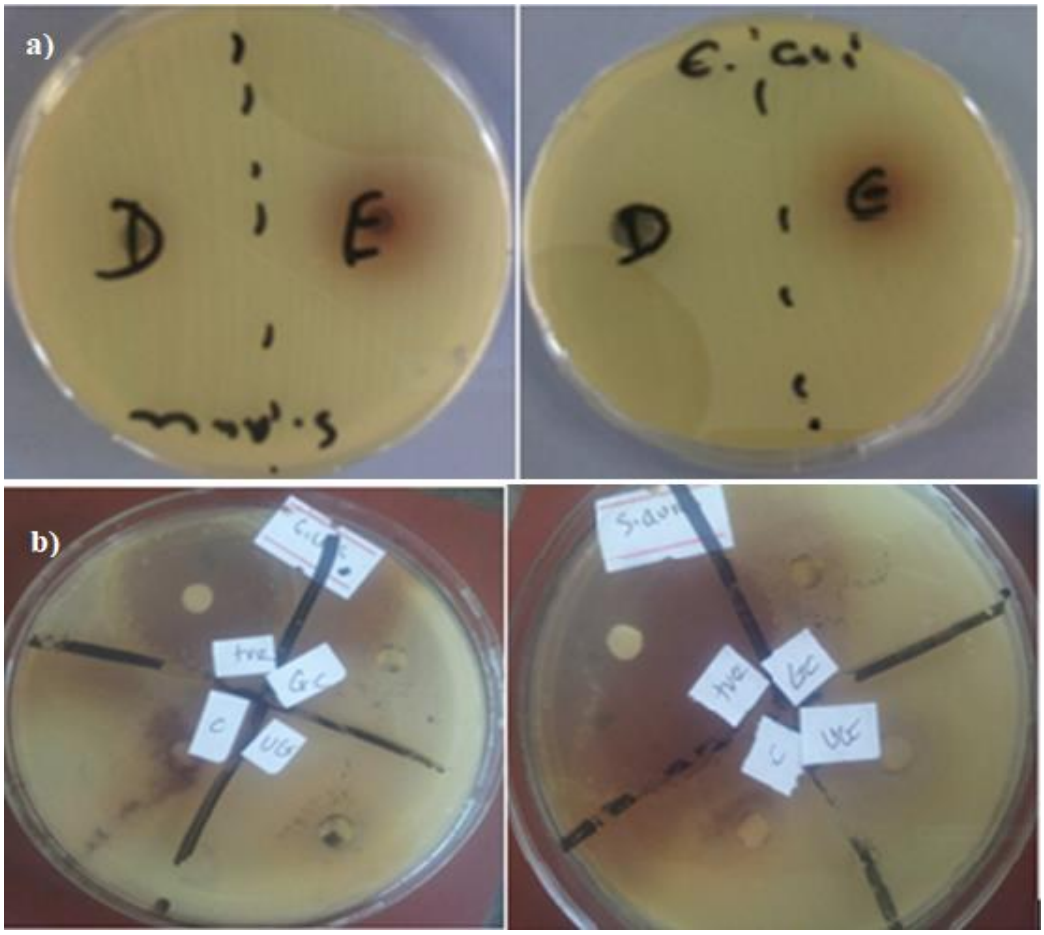

Fig. 7. Antibacterial activity of Co_3O_4 NPs a) at concentration of 25 µg/mL for chemically synthesized (D) and biologically synthesized (E); b) at 50 µg/mL concentration for biologically (GC & UG) and chemically synthesized.

Table 1. Zone of inhibition (mm) of biologically (E) and chemically (D) synthesized Co_3O_4 NPs using 25µg/ml.

S.No	Bacteria	Concentration	+ve control	Green	Chemical
		25µg/ml	ciprofloxacin	(E)	(D)
1	*E.coli*		14.5	10.5	8.8
2	*S.aureus*		14	8.3	8.11

Table 2. Zone of inhibition (mm) of biologically (GC & UG) and chemically (C) synthesized Co_3O_4 NPs using 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

to relatively higher surface area of NPs to volume ration in case of green synthesized ones.

Where GC refers to green synthesized and calcined Co_3O_4 NP while UG refers to green synthesized and uncalcined Co_3O_4 NP, and C refers to chemically synthesized Co_3O_4 NP.

The antibacterial activity of chemically synthesized Co_3O_4 NPs is relatively low as it were confirmed through the measurement of the zone of inhibition, due to the low concentration of ROS and surface area of this nanoparticles compared to those synthesized in biological method. Fig.7 shows the zone of inhibition of the synthesized Co_3O_4 nanoparticles against the two bacteria strains.

As reported by [14, 26-29] the production of ROS is used to break the bacterial cell membrane and as a result, as the superoxide radical production increased, ROS production also increased and results in the enhanced antibacterial application. The antibacterial activity of the synthesized Co_3O_4 nanoparticles in this work is found to be within the range of the previously reported works [29].

CONCLUSION

In the present work Co_3O_4 nanoparticles were synthesized biologically in the presence of Phytolacca dodecandra leaf extract and through chemical methods using co-precipitation method and characterized. The average crystalline size and band gab energy were found to be 10.79 and 11.9 nm, 3.35 and 3.18 eV for the biologically and chemically synthesized Co_3O_4 NPs, respectively. SEM/EDS analysis confirms the spherical shape and morphology of Co_3O_4 nanoparticles, as well the result also confirms the absence of any impurities and foreign materials on its EDS image. The Phytolacca dodecandra extract mediated synthesized Co_3O_4 NPs exhibited considerable antimicrobial activity against both gram negative and gram-positive bacteria strains as compared to the Co_3O_4 NPs obtained via co-precipitation protocol. Based on the present findings, it can be conclude that Co_3O_4 nanoparticles synthesized though the presence of Phytolacca dodecandra extract have shown better antibacterial activity even at low concentration NPs (25µg/ml), with zone inhibition of 10.5 mm against E.Coli, and 8.3 mm against S. aureus than chemically synthesized Co_3O_4 nanoparticles with corresponding zones of inhibition of 8.8 mm and 8.11 mm for both Gram negative and Gram positive human pathogenic bacterial strains respective due to the enhanced production of reactive oxygen species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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