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RESEARCH ARTICLE

Phyto-fabrication of Cobalt Oxide Nanoparticles from *Ocimum Gratissimum* L. Leaf and Flower Extracts and their Antimicrobial Activities

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Abstract:

Background:

Phyto-fabrication of nanoparticles has gained attention in recent times owing to its simple mode, cost-effective and eco-friendly nature.

Objective:

Hence, the present study aimed to synthesize cobalt oxide nanoparticles from methanol extracts of *Ocimum gratissimum* flower and leaf and to evaluate their antimicrobial action towards pathogenic bacteria and fungi.

Methods:

Phyto-fabrication of Cobalt nanoparticles (CoONPs) was achieved using cobalt chloride hydrate solution as a precursor. Characterization of fabricated CoONPs was performed using Ultra Violet-Visible spectrometry (UV-Vis), X-ray diffractometer (XRD), and Fourier Transform-Infrared spectroscopy (FTIR). The antimicrobial property was tested against two bacteria (*Staphylococcus aureus* and *Escherichia coli*) and two fungi (*Cryptococcus albidus* and *Candida globasa*) by agar disc diffusion technique and by measurement of Minimum Inhibitory Concentration (MIC).

Results:

Initial confirmation of CoONPs synthesis was observed by the colour change from light pink to reddish pink. Further, the UV-Vis spectrophotometry validated the CoONPs synthesis with a peak at 509 nm. The XRD authenticated the crystal nature of CoONPs synthesized using flower extract at 2θ angles with an average size of 54.9 nm and CoONPs fabricated using leaf extract at 2θ angles with an average size of 55.02 nm. The FTIR spectroscopy confirmed the functional groups present in plant extracts, which are believed to reduce and stabilize CoONPs. The findings of antibacterial activity showed that CoONPs synthesized using leaf showed a higher inhibition zone against *E. coli* (20.00 ± 2.00 mm) than *S. aureus*. Relating to fungi, the CoONPs fabricated using flower extract displayed significantly the highest activity against *C. albidus* (28.67±0.57 mm) and *C. globasa* (25.0 ± 0.00 mm). The lowest MIC was observed against *S. aureus* (MIC 7.5 µg/ml). For fungi, the smallest MIC was found against *C. albidus* (MIC 2.5 µg/ml).

Conclusion:

The current research established the efficacy of the phytochemical constituents of leaf and flower extracts of *O. gratissimum* for the enhancement of the effectiveness of CoONPs against both the tested bacteria and fungi.

Keywords: Antifungal activity, Biosynthesis, Characterization, Cobalt Oxide nanoparticles, FTIR, XRD.

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1. INTRODUCTION

The bacterial and fungal resistance to conventional antibiotics and medicines has persistently emerged. Hence, there's a dire need to develop alternative antimicrobial agents

that could end the menace of bacteria and fungi that are resilient to existing antibiotics [1]. To overcome this, there are promising alternatives to combat pathogenic microbes through nanotechnology by synthesizing nanoparticles [2]. Amidst various metal nanoparticles, cobalt oxide NPs are known to sustain astonishing physical, chemical, and optical properties, at extreme circumstances [3]. Cobalt, a constituent of vitamin B12, is useful for the treatment of anemia since it initiates the

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red blood cells formation [4]. Moreover, it is used in the arena of Nanosensors and Nanoelectronics owing to its unique optical, magnetic, catalytic, and electrical properties [3, 5]. Various states of oxidation or multivariate states such as Co²⁺, Co³⁺, and Co⁴⁺ can be exhibited by cobalt, which is responsible for its diverse states of spin in their oxide forms, *i.e.*, low, intermediate, and high. This feature allows cobalt to be used for different industrial purposes [6].

The biomedical industry has embraced the applications of CoONPs that are used as antimicrobials, for heartburn problems, regeneration of bone, drug delivery, as well as for cancer treatment [7]. Many approaches, such as the physical, chemical, and biological techniques, are reported for the fabrication of CoONPs [8]. Among the prevailing methods, chemical methods are capable of synthesizing great amounts of nanoparticles with controlled distribution and size in a comparatively short time [1]. However, the usage of chemicals with high toxicity, the production of harmful wastes, and high energy consumption are the demerits. Similarly, physical methods such as the microwave technique and cluster beam deposition, with the contribution of high temperature, high pressure, and radiation, are also used for the fabrication of nanomaterials [9]. Due to this, methods utilizing biological materials are the better replacement for the above-said methods for the fabrication of nanoparticles [10].

Nowadays, various microorganisms and plant extracts are used effectively for the fabrication of metal NPs either intracellularly or extracellularly [10, 11]. The bio-method is a bottom-up approach and requires less energy, less wastage of ingredients and a proper control of reagents. Hence, nowadays efforts are made to evaluate the antibacterial effects of bio-synthesized nanoparticles and the synergistic effect of both the metal and the plant extracts [12]. Above all, phyto-synthesis of nanoparticles is highly preferred as this method is cost-effective, efficient, eco-friendly, and involves a comparatively smaller amount of energy than physical and chemical approaches [13]. The nanomaterials fabricated by biosynthesis are well known for their smaller size, dynamic movement, and reduced surface area [14]. Moreover, they exhibit fewer side effects than the synthetic antibiotics [13]. Bio-fabrication of CoONPs has been given utmost interest due to economical production and various electrical, mechanical and optical properties of the synthesized cobalt NPs [12]. Above all, CoONPs are less toxic at low concentrations in the human body and have potent activities towards pathogenic microbes at lower doses. Recent research established the biosynthesis of CoONPs and their efficacy against microbial pathogens [15 - 18]. The sweet basil (*O. gratissimum* L.) is a medicinal plant endowed with active components that are useful against various pathogenic conditions. Moreover, studies on the synthesis and antimicrobial activities of nanoparticles in Ethiopia are in the juvenile stage. Therefore, this study was carried out to investigate the phyto-fabrication of CoONPs from leaves and flowers of *O. gratissimum* and assess their antimicrobial activities.

2. MATERIALS AND METHODS

2.1. *O. Gratissimum* Leaf and Flower Extracts Preparation

The fresh leaves and flowers of *O. gratissimum* were collected from the locality in Damota village, Haramaya

district, Ethiopia. The fresh *O. gratissimum* leaves and flower materials were washed 3 times with water to remove dust and were dried before being powdered. After that, the dried leaves and flower materials were powdered using a homogenizer. Then 20 g of powder samples were added to 200 ml of methanol in two flasks, separately. Then, they were kept in a water bath shaker for 8. Thereafter, the crude extracts with solvent were filtered with the help of Whatman No.1 filter paper to remove particulate matter and to obtain the methanol extract of *O. gratissimum*. Freshly prepared methanol extract of *O. gratissimum* leaves and flowers were sealed with aluminum foil. Then, it was stored at 4 °C for future use.

2.2. Phyto-fabrication of CoONPs

The CoONPs fabrication was performed using Cobalt chloride hexahydrate (CoCl₂ 6H₂O) as a precursor [19]. Aqueous solution 1.06 x 10⁻² M of CoCl₂ 6H₂O was prepared in 250 ml Erlenmeyer flask, and 20 mL of plant parts extracts of *O. gratissimum* (leaves, flowers) were separately added drop wisely to 250 mL conical flask containing 100 ml of 1.06 x 10⁻² M CoCl₂ 6H₂O solution for the reduction of Co⁺ ions. The complete mixture was kept on magnetic stirring at 70C temperature. Suitable controls were maintained throughout the conditions of the experiments. The mixture was then centrifuged at 4500 rpm for 15 min. The precipitates obtained were kept in an oven (500 °C) for 2h, grounded, and subjected to characterization by different spectrophotometric analyses.

2.3. Characterization of CoONPs

2.3.1. Visual and UV-Vis spectroscopy examination

The phyto-synthesis of CoONPs using *O. gratissimum* (leaves, flowers) in the medium was initially confirmed by the changes in color from a light pink to reddish brown. Besides, characterization of the formation of biosynthesis of CoONPs and bio-reduction of the Co⁺ ions in solution was monitored by UV-visible spectroscopy (Model 6705) at the wavelength of 200-700 nm range. The absorption wavelength at maximum was measured by keeping each aliquot NP material in a quartz cuvette (1 x 1 cm) functioning at a resolution of 1 nm, using distilled water as the blank. The wavelengths of the Co nanoparticles were observed based on the SPR formed for the metal, which is produced by clear oscillation of electrons generated by a light source [20].

2.3.2. Characterization by XRD

Characterization by XRD was done using X-ray diffractometer (XRD-7000, SHIMADZU, Japan) with monochromatic radiation (Cu K α ; λ = 1.5406 Å) functioned at 30 mA and 40 kV at a pattern of 2 θ angle. The range of 20°–80° was used for scanning and the images acquired were equated with the Joint Committee on Powder Diffraction Standards (JCPDS) library to determine the crystal structure [20]. The size of the NP grain was calculated with the help of Scherer's Formula as follows:

$$D = (0.9\lambda) / \beta X \cos\theta$$

Where, D denotes Average Crystal size, β denotes Line broadening (radians), θ denotes Bragg angle and λ denotes wavelength of X-ray.

2.3.3. FTIR Characterization

The FTIR analysis was performed by Fourier Transform Infrared Spectrophotometer (model: Perkin Elmer Frontier FTIR) [21]. The analysis was done to detect the chemical groups that might be responsible for the capping and reduction of the Cobalt bulk materials to NPs at a wavelength of 4000-400 cm^{-1} range. For this, the dried sample was added with Potassium Bromide (KBr) and it was made to 1 mm thickness which was appropriate for FTIR analysis.

2.4. Antimicrobial Activity of Phyto-fabricated CoONPs

The antimicrobial effect of Cobalt NPs was investigated using disc diffusion and MIC methods [22]. In the disc diffusion method, three concentrations (15, 25 and 35 $\mu\text{g/ml}$) were used against two different bacterial species (gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*) and two fungi (*Cryptococcus albidus* and *Candida globasa*). The two test bacteria were procured from EPHI, the Ethiopian capital city of Addis Ababa. The two fungal pathogens were obtained from the Plant Pathology lab at Haramaya University. The bacterial and fungal stains were sub-cultured and maintained on Nutrient Agar and Potato Dextrose Agar (PDA), respectively. The bacterial culture was adjusted to the turbidity of 0.5 McFarland standard solutions (1.5×10^8 bacteria/ml). Henceforth, the bacterial and fungal cultures were incubated for 18-24 h at 37 °C and 72 h at 27 °C respectively. For the determination of minimum inhibitory concentration (MIC), broth dilution method was employed.

2.4.1. Agar Disc Diffusion Test

For antimicrobial activity of *O. gratissimum* synthesized CoONPs by disc diffusion method, bacterial and fungal cultures (200 μl) were uniformly streaked as lawns on the respective medium. Ten μl of the CoONPs at three different concentrations (15, 25 and 35 $\mu\text{g/ml}$) were loaded into

Whatman No. 1 paper discs (6 mm), and the discs were accommodated on the lawns of bacterial and fungal cultures. Amoxicillin (10 $\mu\text{g/ml}$) and ketoconazole (10 $\mu\text{g/ml}$) were utilized as positive controls for bacteria and fungi, respectively whereas, methanol was used as a negative control. The Petri plates were then incubated for 24 hr at 37°C for bacterial strains and 28°C for 72 hr for fungal strains. After incubation, the zones of inhibition of the pathogens were measured.

2.4.2. MIC Determination

For MIC determination, the lowest concentrations of CoONPs that displayed good antimicrobial activity during the agar diffusion method were selected for the serial dilution method and the method of Adera *et al.* [23] was followed with slight modifications. In brief, two-fold dilution of 15 $\mu\text{g/ml}$, 7.5 $\mu\text{g/ml}$, 3.75 $\mu\text{g/ml}$, 1.875 $\mu\text{g/ml}$, and 0.9375 $\mu\text{g/ml}$ from 15 $\mu\text{g/ml}$ of CoONPs. Nutrient broth and potato dextrose broth (2 ml) were added for bacteria and fungi respectively into test tubes and CoONPs (0.1 ml) were mixed with the nutrient broth and potato dextrose broth. Afterwards, Inoculums (0.1 ml) of each pathogen were distributed into the test tubes comprised of broth suspensions and the CoONPs. The test tubes were then closed using aluminum foil and incubated at 37°C for 24 hrs in the case of bacteria and 27°C for 72 hrs for fungi. Finally, the tubes were monitored for presence or absence of the visible growth of the microbes. The least concentration at which no visible of the microbes noted was the value of MIC. The MIC test was done in triplicate for each pathogen.

2.5. Data Analysis

The experimental data in Tables 1 and 2 (supplementary material file) was first entered into Microsoft Excel, and a one-way analysis of variance (ANOVA) was used from 9.2 software package (SAS version), and the statistical results were reported as a mean \pm standard deviation. Using Post Hoc' DMRT, significant differences were specified at $P < 0.05$.

Table 1. Average crystalline sizes CoONPs from flower and leaf extracts of *O. gratissimum*.

Plant Extract	2 θ (deg.)	FWHM	D (nm)
Flower	40.90221	0.1567	54.90913
Leaf	39.77866	0.156738	55.02112

Table 2. Antimicrobial activity of biosynthesized CoONPs from *O. gratissimum*.

Pathogens	Part of the Plant Used	The Concentration of Biosynthesized Nanoparticles ($\mu\text{g/ml}$)			Amoxicillin (10 $\mu\text{g/ml}$)	Met
		15 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	35 $\mu\text{g/ml}$		
<i>E. coli</i>	Leaf	11.33 \pm 1.15 ^{cd}	14.33 \pm 0.57 ^{dc}	20.00 \pm 2.00 ^{cb}	19.67 \pm 0.57 ^{aA}	-
<i>E. coli</i>	Flower	11.67 \pm 0.57 ^{cd}	13.67 \pm 0.57 ^{dc}	19.67 \pm 2.51 ^{bc}	20.5 \pm 1.30 ^{aA}	-
<i>S. aureus</i>	Leaf	10.33 \pm 0.57 ^{bd}	12.33 \pm 0.57 ^{dc}	16.00 \pm 0.00 ^{db}	18.83 \pm 1.04 ^{aA}	-
<i>S. aureus</i>	Flower	10.00 \pm 0.00 ^{bd}	12.33 \pm 0.57 ^{dc}	15.00 \pm 1.00 ^{db}	19.83 \pm 1.32 ^{aA}	-
-	-	-	-	-	Ket (10 $\mu\text{g/ml}$)	Met
<i>C. albidus</i>	Leaf	21.00 \pm 1.00 ^{bd}	23.67 \pm 0.57 ^{ac}	28.33 \pm 1.52 ^{ab}	30.5 \pm 0.57 ^{aA}	-
<i>C. albidus</i>	Flower	18.67 \pm 2.30 ^{bc}	25.33 \pm 0.57 ^{ac}	28.67 \pm 0.57 ^{aA}	31.17 \pm 1.04 ^{aA}	-
<i>C. globasa</i>	Leaf	13.67 \pm 1.50 ^{bcd}	17.67 \pm 0.57 ^{cb}	23.67 \pm 2.05 ^{ba}	29.83 \pm 0.16 ^{aA}	-
<i>C. globasa</i>	Flower	14.67 \pm 0.5 ^{bc}	21.3 \pm 2.3 ^{bb}	25.0 \pm 0.00 ^{ba}	28.0 \pm 1.00 ^{aA}	-

Note: All the given values are triplicate; The means \pm SD followed by the same letter within a column were not significantly different at $P < 0.05$ and Mean values with different letters show a significant difference at $P < 0.05$; Small letters: significant within the column; Capital letters: significant across the rows. - = No activity, Met: Methanol, Ket: Ketoconazole.

3. RESULTS

3.1. Characterization of Synthesized CoONPs

3.1.1. Visual Observation of Synthesized CoONPs

The biosynthesis of CoONPs from methanol extract of leaves and flowers of *O. gratissimum* was, to begin with, affirmed by visual perception of the change of the color amid response (Fig. 1). The aqueous solution of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ was shown light pink, which was later changed to reddish brown as we added extract. This color change demonstrated the formation of CoONPs, whereas controls (without Co^{+} particles) showed no alteration in color.

3.1.2. Characterization based on UV-visible Spectrophotometry

The brown-colored samples of CoONPs synthesized using both the leaves and flower extracts of *O. gratissimum* were

subjected to UV-Spectrophotometry in the range of 200-700 nm. Fig. (2A and B) indicated the absorbance crest obtained for phyto-fabricated CoONPs.

3.1.3. Characterization based on (XRD)

The XRD analysis of the phyto-fabricated Cobalt oxide NPs from the leaves and flower extracts of *O. gratissimum* was recorded and a typical XRD pattern is shown in Fig. (3A and B). The high diffracted intensities were obtained around $2\theta = 16.08^\circ, 20.7^\circ, 31.3^\circ, 33.04^\circ, 37.82^\circ, 38.88^\circ, 39.32^\circ, 42.28^\circ, 43.58^\circ, 50.56^\circ, 54.64^\circ, 55.24^\circ, 67.98^\circ$ corresponding to the plane 110, 020, 111, 220, 120, 130, 201, 422, 040, 131, 400, 420, 440 for A and $16.06^\circ, 20.78^\circ, 31.32^\circ, 32.46^\circ, 33.00^\circ, 33.82^\circ, 37.8^\circ, 38.84^\circ, 39.28^\circ, 42.26^\circ, 43.56^\circ, 55.24^\circ, \text{ and } 58.04^\circ$ corresponding to the plane 110, 020, 111, 220, 120, 130, 201, 422, 040, 131, 400, 420, 440 for B. Bragg reflections of Face centered (FCC) crystalline phases of the Cobalt NPs and they are in agreement with the pattern no. 96-152-4727.



Fig. (1). The color change represents the formation of cobalt oxide nanoparticles.

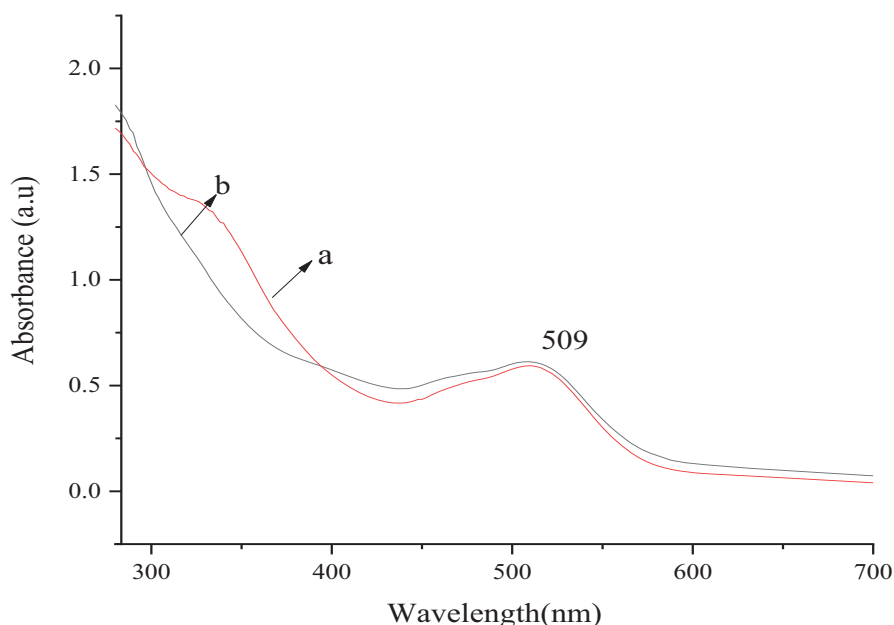


Fig. (2). UV-Vis spectra of biosynthesized CoONPs by using methanol extracts of *O. gratissimum* (A) leaves and (B) flowers.

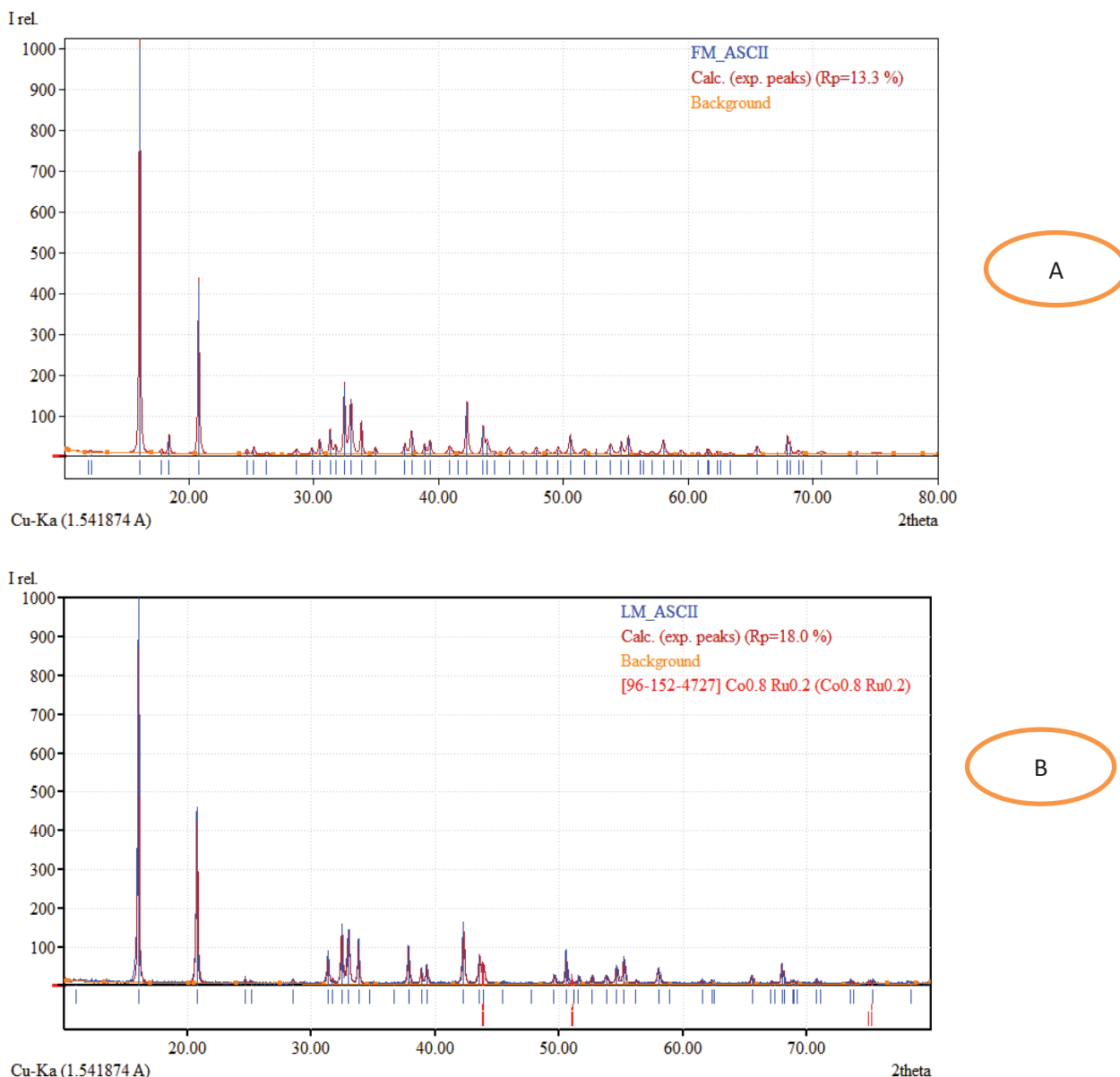


Fig. (3). XRD pattern of biosynthesized cobalt oxide nanoparticles from *O. gratissimum* (A) from the flowers part and (B) from the leaf part.

The XRD peaks measured were found to match with the Cobalt's standard patterns. The sharp peaks authenticated the crystal nature of CoONPs. The average crystal size of fabricated NPs with the flower extract was measured to be 55.02 nm based on the equation of Debye-Scherrer. The average size of 54.90 nm was observed for CoONPs synthesized with the leaf extract as it is shown in Table 1.

3.1.4. Characterization by Fourier Transformation Infrared (FTIR) Spectroscopy

Fig. (4a and b) showed the FTIR result of CoONPs recorded inside the wave number run 500-4500 cm^{-1} . The FTIR spectra of CoONPs from leaves and flowers extract of *O. gratissimum* observed different bands at 667, 1050, 1386, 1425, 1625, 3439 and 478, 669.79, 778, 1051, 1341.9, 1419.9,

2170, 3439 cm^{-1} respectively. These compared to Co-O bowing mode of CoO in Co was Co^{2+} and was tetrahedrally coordinated.

FTIR spectra of leaves-mediated biosynthesized cobalt oxide nanoparticles showed the two-solid band 3439 cm^{-1} and 1,341.9 cm^{-1} . The broadband at 3439 cm^{-1} was due to O-H extending in alcohols and phenols or N-H extending of amines. The top at 1,341.9 cm^{-1} was compared to the essential and auxiliary amine bunches of N-H bowing vibrations of proteins and characteristic of -C=O carbonyl bunches. The crests at 2,170 cm^{-1} related to alkynyl $\text{C}\equiv\text{C}$ stretch and crests at 1,051 cm^{-1} may well be attributed to -C-O , -C-O-C extending, and C-N stretch of aliphatic and aromatic amine. The pick at 669.5 cm^{-1} indicated the nearness of Co-O vibrations in which Co is Co^{2+} and is tetrahedrally coordinated.

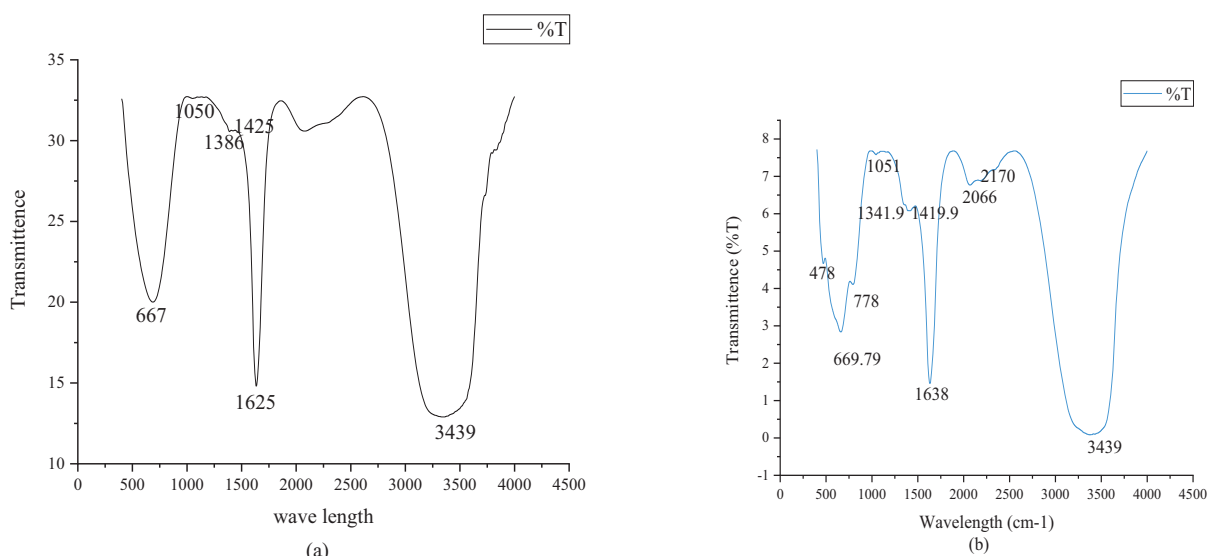


Fig. (4). FTIR spectra of synthesized cobalt oxide nanoparticles from *O. gratissimum* (a) from leaf part and (b) from flowers part.

3.2. Antimicrobial Activity of CoONPs

3.2.1. Antibacterial Studies

Antibacterial activity of biosynthesized CoONPs from *O. gratissimum* leaf and flower extracts at three different concentrations is recorded in Table 2. The findings revealed that the antibacterial action of CoONPs was concentration-dependent. The results exhibited higher activity by leaf-fabricated CoONPs against *E.coli* followed by *S. aureus* with a zone of inhibition of 20.00 ± 2.00 mm and 16.00 ± 0.00 mm, respectively. The flower extract-fabricated CoONPs displayed significantly ($P < 0.05$) higher activity against *E.coli* (19.67 ± 2.51 mm) than *S. aureus* (15.00 ± 1.00 mm). The antibacterial potential of CoONPs was found to be significantly ($P < 0.05$) less than that of the standard antibiotic amoxicillin. Methanol, as a negative control, did not show any antibacterial activity.

3.2.2. Antifungal Activity of Biosynthesized CoONPs

The results of *in vitro* antifungal activity of CoONPs fabricated using leaves and flowers of *O. gratissimum* against *C. albidus* and *C. globasa* are illustrated in Table 3 and Fig. (5). The CoONPs exhibited significant antifungal activity against the tested fungi (*C. albidus* and *C. globasa*). The leaf-fabricated CoONPs established the highest antagonism towards *C. albidus* and *C. globasa* with the inhibition diameter of 28.33 ± 1.52 and 23.67 ± 2.05 mm respectively at the concentration of 35 $\mu\text{g/ml}$. The flower-fabricated CoONPs demonstrated the highest activity against *C. albidus* and *C. globasa* with a zone of inhibition of 28.67 ± 0.57 mm and 25.0 ± 0.00 mm respectively at the concentration of 35 $\mu\text{g/ml}$. The antifungal potential of ketoconazole against the tested fungi was found to be significantly higher than the activity ($P < 0.05$) of CoONPs with an inhibition zone range of 31.17 ± 1.04 mm to 25.00 ± 1.00 mm *C. albidus* and *C. globasa* against respectively.

Table 3. Minimum inhibitory concentration (MIC) of CoONPs from *O. gratissimum*.

Pathogens	Part of the Plant	Amoxicillin/Ketoconazole ($\mu\text{g/ml}$)	MIC of CoONPs ($\mu\text{g/ml}$)
<i>S. aureus</i>	Leaves	5.00 \pm 0.00	12.50 \pm 4.33
	Flowers		15.00 \pm 0.00
<i>E. coli</i>	Leaves	5.00 \pm 0.00	7.50 \pm 0.00
	Flowers		10.00 \pm 4.33
<i>C. albidus</i>	Leaves	1.75 \pm 0.00	2.50 \pm 1.08
	Flowers		2.50 \pm 1.08
<i>C. globasa</i>	Leaves	2.5 \pm 0.00	3.75 \pm 0.00
	Flowers		3.75 \pm 0.00

Note: All values are triplicate means + SD, MIC: Minimum inhibitory concentration (MIC).



Fig. (5). Inhibition zone of bacteria test pathogens on Petri plates.

3.2.3. The Minimum Inhibitory Concentrations of CoONPs

Furthermore, the antimicrobial potential was evaluated to determine the MIC of phyto-fabricated CoONPs against the tested pathogenic microorganisms using the serial dilution method. The MIC values of the biosynthesized CoONPs from *O. gratissimum* leaf and flower methanol extract are recorded in Table 3.

In the current investigation, the lowest MIC (7.5 $\mu\text{g/ml}$) and the highest antibacterial potential of the CoONPs was noted against *S. aureus*, while a lesser potent antibacterial activity with MIC value (15 $\mu\text{g/ml}$) was observed towards *E. coli*. Besides, the highest antifungal effect by CoONPs was found against *C. albicans* (MIC 2.5 $\mu\text{g/ml}$) and the lowest antifungal activity against *C. globosa* with a MIC value of 3.75 $\mu\text{g/ml}$.

4. DISCUSSION

As the research on nanoparticles is fresh-faced stage in Ethiopia, the current work was carried out on phyto-assisted fabrication of CoONPs using the flower and leaf extracts from *O. gratissimum*. Post-synthesis, the CoONPs were subjected for validation using characterization techniques and antimicrobial activity against two bacterial and two fungal pathogens.

The initial validation of CoONPs was observed by light pink to reddish brown color change in the reaction mixture which suggested that the precursor was reduced to Cobalt ions (Co^{2+}) with the help of phyto-components of the flower and leaf extracts. The reddish brown color formation was attributed to the occurrence of External Plasmon Resonance [24]. Further, the UV spectroscopic examination for the fabricated CoONPs disclosed a 590 nm peak that demonstrated a nearness of nanoparticles within the response blend. The CoONPs would generally display a band of absorption at 350-550 nm range which is accredited to Surface Plasmon Resonance of Co

nanostructure [25]. The present results endorse the preceding absorption spectral profile [25]. The alteration in the color of the response blend was moreover due to the surface Plasmon resonance that provides a helpful sign of the arrangement of CoONPs.

With regard to the characterization of CoONPs by XRD, the planes detected suggested that the phyto-fabricated CoONPs were crystal in nature. The scattering peaks at 2θ angle were indexed at different planes as shown in the results. The results obtained in our study were in concomitant with the results reported by Khalil *et al.* [26]. Additionally, certain unassigned peaks found at the angle of 2θ might be accredited to impurities in the sample or attributed to crystals of organic contents [27]. Albeit the paramount role of electron microscopy in the determination of the size and shape of the CoONPs, the lack of facilities hampered us to perform the same.

The FTIR examination was utilized to decide the biomolecules that are responsible for capping and proficient stabilization of cobalt oxide nanoparticles synthesized utilizing *O. gratissimum* leaves, and flowers extract. FTIR can identify functional groups present inside the nanoparticles synthesized using plant and its parts extracts. The FT-IR spectra obtained were recorded for CoONPs between 4500 - 500 cm^{-1} in KBr pellets at the resolution of 4 cm^{-1} . The wide band at 3439 cm^{-1} was the characteristic crest of a hydroxyl (O-H) bunch of phenolic compounds [28]. The absorption band observed at 1386 cm^{-1} was owing to the C-O extend whereas the absorption band at 1625 cm^{-1} was because of C=C and C=O extension of benzene and amide-I linkage. The exceptionally solid band at 1050 cm^{-1} emerged from C-O-C symmetric extending, C-O in carbohydrates, ester, liquor, ether, and C-O-H twisting vibrations of protein in *O. gratissimum*. Retention crests within the FTIR range of CoONPs showed up at 669 cm^{-1} , compared to the metal-oxygen extending vibration. The observed bands

may be considered for the proximity of various compounds such as phenolic, flavonoid, and carboxylic compounds. All of these asserted that methanol-soluble phytochemicals displayed within the leaves and flower extract of *O. gratissimum* can achieve reduction and stabilization of the cobalt nanoparticles [29]. Huang *et al.* [30] reported, on MNP blend, the useful bunches of alkaloids, anthracenes, flavones, and, such as $-C-O-C-$, $-C=C-$, $C-O-$, and $-C=O-$.

The present study highlighted the potential of CoONPs against *E. coli* and *S. aureus*. *S. aureus* is considered to be the reason for the infection of soft tissues, bacteremia, and pneumonia, and has been stated to be resistant to methicillin [31]. The inhibitory potential of the CoONPs against the bacteria tested was owing to the large surface area to volume ratio of the nano-sized particles that enhanced their interaction with the microorganisms and caused a change in their permeability. This permeability allowed the NPs to enter into bacterial cells and directly kill them [32]. The CoONPs could directly bind to the bacterial DNA to induce cell death of the bacteria. The reduced CoONPs were capable of binding directly with the bacterial DNA to encourage cell death of the bacteria [33]. Similar studies were found to exhibit the effect of CoONPs against gram (-) *E. coli* and gram (+) *S. aureus* [17, 34 - 36]. The CoONPs at all three concentrations exhibited higher inhibition zones against *E. coli* than against *S. aureus*. A similar trend was reported in previous research by [37]. The reason for the distinctive sensitivities of gram (-) and gram (+) bacterial pathogens to the biosynthesized CoONPs could be due to the morphological contrasts between these microorganisms [38]. Besides, the positive ions of Cobalt NPs {Co (2+)} interact directly with the negatively charged liposaccharides found on the cell surface of Gram-negative bacteria that lead to the inhibition of enzyme biosynthesis [39]. The current study also revealed that CoONPs displayed antifungal activity against the fungi *C. albidus* and *C. globasa*. The effect of CoONPs against the fungi showed that the Cobalt NPs were able to stabilize therapeutic components from plant extracts that could penetrate extracellular polymeric substances (EPS) to kill fungal cells [40].

CONCLUSION

The phyto-fabrication of CoONPs using plant extracts assured a quick, less-toxic, and eco-friendly path. The current study showed the action of methanol extracts in *O. gratissimum* leaves and flowers for the fabrication, capping, and stability of CoONPs. The phyto-fabricated CoONPs were characterized effectively with UV-Vis spectra, FT-IR and XRD methods. Effective antibacterial action of the phyto-fabricated CoONPs towards *E. coli* and *S. aureus* and antifungal activity against *C. globasa* and *C. albidus* were detected. Based on the observed findings, the current research would be an attempt to further carry out investigations in the future on the efficacy of CoONPs synthesized using Ethiopian medicinal plants against broad spectrum of microbial pathogens that exhibit resistance to conventional antimicrobial drugs. Future studies are also recommended to explore medicinal plants from Ethiopia for phyto-synthesis of other nanoparticles to combat microbial infections.

AUTHORS' CONTRIBUTION

Mohammed Abdi contributed to Initiation and design of the study, Lab experiment, and data analysis; Zekeria Yusuf contributed to lab experiment, data collection, and write-up of the document; J.M. Sasikumar: wrote the manuscript, and contributed to analysis and interpretation of data. All authors contributed to drafting the article and revising it critically for important intellectual content.

LIST OF ABBREVIATIONS

CoONPs	= Cobalt nanoparticles
UV-Vis	= Violet-Visible spectrometry
XRD	= X-ray diffractometer
MIC	= Minimum Inhibitory Concentration
JCPDS	= Joint Committee on Powder Diffraction Standards
KBr	= Potassium Bromide
PDA	= Potato Dextrose Agar

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No humans/animals were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The CoO nanoparticles manuscript data file used to support the findings of this study is included within the supplementary information file(s).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIALS

The CoO nanoparticles manuscript data files used to support the findings of this study are included within the supplementary information file(s).

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