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Biosynthesis of cobalt oxide (Co_3O_4) nanoparticles using plant extract of *Camellia sinensis* (L.) Kuntze and *Apium graveolens* L. as the antibacterial application

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ABSTRACT

In this work, we prepared cobalt oxide nanoparticles using Celery stalks and green tea leaves extract. The synthesized cobalt-oxide NPs were characterized using X-Ray diffraction. This showed the highest peak and top control (222) at (38.18 degree) with regard to *Camellia sinensis* extract and (220) at (30.14 degree) for *Apium graveolens* extract, Field Emission scanning electron microscopy (EF_SEM) at the range of 21-72 nm, revealed the highly uniform shape of particles, while UV-Visible spectroscopy techniques recorded the highest value of the absorptive at 230 nm and the energy band gap to be 3.55 eV for *Camellia sinensis* and 225 nm and energy band gap 3.85 eV at *Apium graveolens*, respectively. Our results indicate that the best achievable result in inhibiting bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, comes by using *Camellia sinensis* extract (27-29 mm).

***Keywords*:** Cobalt oxide nanoparticles, *Camellia sinensis*, *Apium graveolens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

1. INTRODUCTION

Nanotechnology is “the design, characterization, devices, production, application of structures, and systems by controlling shape and size at nanometer scale” [1]. Normally, within this range, materials may have properties considerably different from those expected when they

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have larger dimensions. Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nano size objects [2, 3]. Green way so necessary to produce nanomaterials environmentally friendly with a high specification for that plant extracts containing secondary metabolites (ketones, amides, flavones carboxylic acids, phenols) is reducing agent for reaction and stabilizing to synthesize nanoparticles for oxide fuels using plant extracts composing anti-particles for bacteria and this technology featuring, efficient and safe for the environment and inexpensive. Chemical methods involve the reduction of chemicals without extracts [4], procedures electrochemical [5], micro-emulsion, chemical precipitation, chemical vapor condensation, and pulse electrodeposition [6].

They include typical procedures where grown nanoparticles in the middle of a liquid extract contain multiple laboratory reagents, such as sodium hydroxide [7] or hydrazine [8] or methoxy polyethylene glycol [9]. A nanometer (nm) is a billionth of a meter. The bacterial membrane contains sulfur- containing proteins, and the cobalt oxide nanoparticles interact with these proteins in the cell as well as with the DNA. The nanoparticles preferably attack the respiratory chain and cell division, finally leading to cell death. The nanoparticles release cobalt oxide in the bacterial cells enhancing their bactericidal activity [10].

2. MATERIAL AND METHODS

2. 1. Chemical and reagents

In this study, distilled water (DW) was used as a solvent, cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$), (99%), (Reagent World, USA, purity 99.99%), *Camellia sinensis* leaves, *Apium graveolens* stalks and sodium hydroxide were collected from the local market, Baghdad.

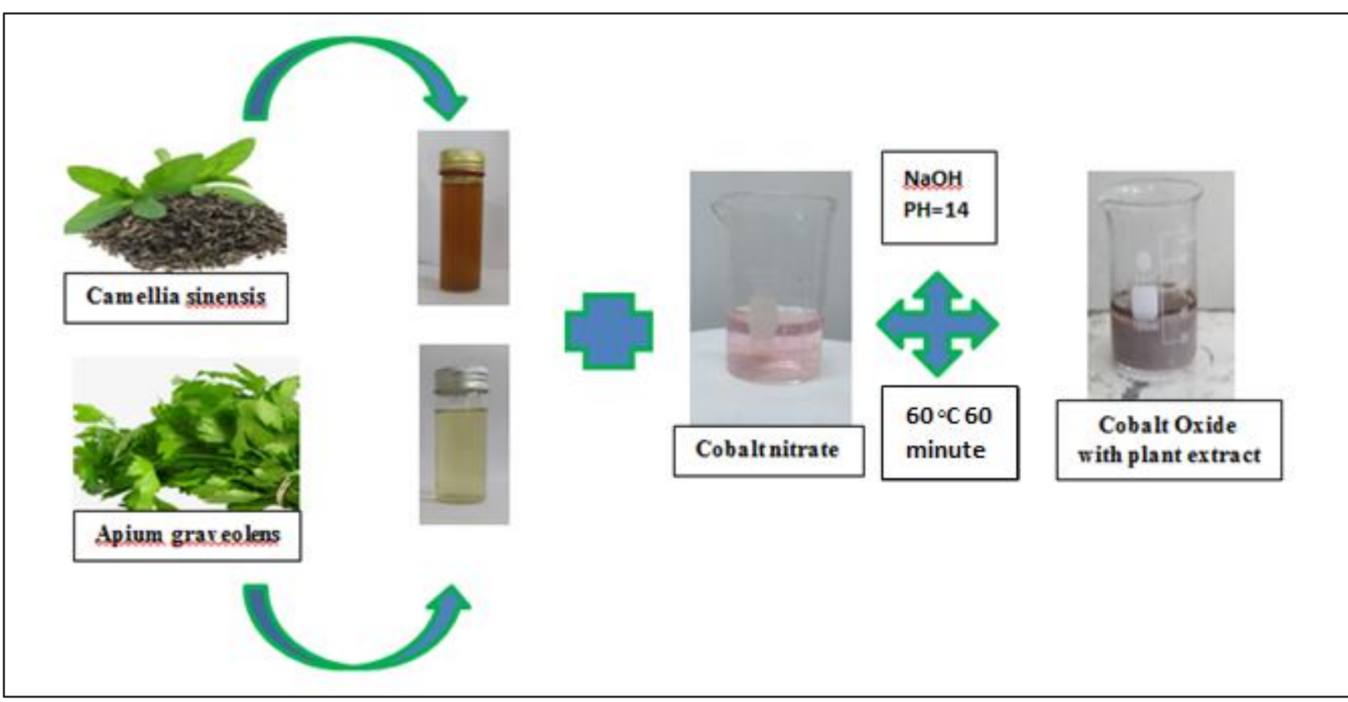

Fig. 1. Synthesis of Co_3O_4 NPs using plant extracts *Camellia* and *Apium* with cobalt nitrate

2. 2. Preparation of stabilizing agent (plant extract)

The Celery stalks (*Apium graveolens*) were sliced into pieces and washed with ultrapure water to remove impurities *Apium graveolens* (30 g) and 200 mL water was homogenized in an electrical grinder. Then the mixture was heated at 80 °C along with continuous stirring, cooled down and filtered. The filtrate (brown color) was collected and used for the synthesis of cobalt oxide NPs, the same procedure was done for green tea leaves.

2. 3. Synthesis of cobalt oxide (NPs)

To the synthesis, the cobalt oxide NPs, freshly extract (30 mL) was added to 0.02 M solution of cobalt nitrate, heated at 85 °C fort 30 min, then sodium hydroxide (pH = 14) was added for 60 minutes. The mixture was kept overnight at room temperature and then centrifuged at 14,000 rpm for 10 min. The obtained precipitates were dried in an oven at 500 °C for 2 h, ground and subjected to characterization. Synthesis of Co_3O_4 NPs using plant extracts and mixing it with cobalt nitrate, and turning it from pink to gray, forming the cobalt oxide. The process of the synthesis of Co_3O_4 NPs with plants is shown in **Figure 1**.

3. RESULTS AND DISCUSSION

3. 1. X-Ray analysis

XRD of Co_3O_4 NPs was done using green tea. The oxide was polycrystalline by specification (JCPDS Card no. 042-1467) and phase structure, cubic shape and didn't show any article other than that extracted, used as search method with the outfit as that successful chemical picas are distinct and no trace of moisture dust concentrations are enlarged, as were appropriate for the sample.

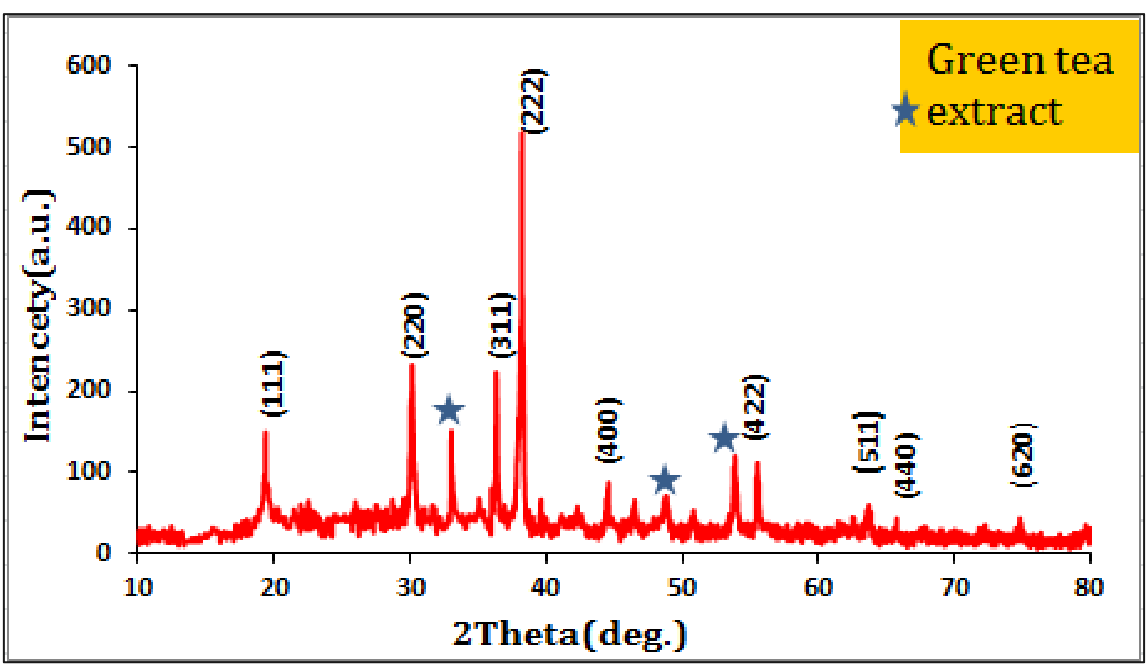

Figure 2. XRD pattern of cobalt oxide nanoparticles using *Camellia sinensis* extract

Number of Bragg values are (111, 220, 311, 222, 400, 422, 511, 440, 620) and celery extract (111, 220, 311, 222, 400, 422, 511, 531) XRD pattern indicates that the cobalt oxide nanoparticles (**Figures 2** and **3**).

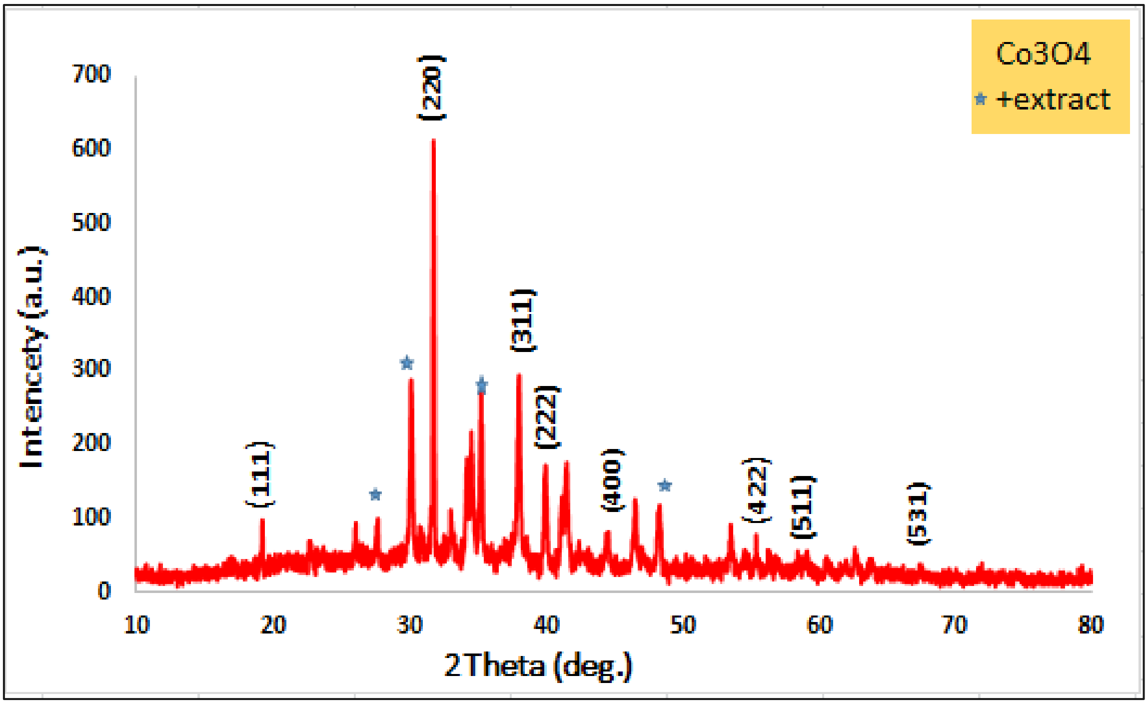

Figure 3. XRD pattern of cobalt oxide nanoparticles using *Apium graveolens* extrac.

The average crystallite size of the cobalt oxide nanoparticles was calculated, using Debye-Scherrer equation):

$$D = 0.9\ \lambda/\beta\ \text{cost}(2\theta) \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where: D is the particle size (in nm), k is a constant equal to 0.94, λ is the wavelength of X-Ray radiation (1.541A), β is the full-width at half maximum (FWHM) of the peak (in radians) and 2 theta is the Bragg angle (in degrees). The average crystallite size was found to be in the range of (21-55 nm), sharp peaks (222) in (2θ = 38 dig.) from *Camellia sinensis* extract and (21-74 nm) sharp peaks (220) in (2θ =31 dig.) from *Apium graveolens* extract.

3. 2. FE-SEM analysis

The FE-SEM images of cobalt oxide nanoparticles are shown in **Figures 4** & **5**. The morphology of the nanoparticles indicates irregular, small particles and stakes of various sizes.

3. 3. UV-visible spectroscopy

This was UV-vis spectroscopy which is frequently used to characterize synthesized nanoparticles. **Figure 6** shows the UV-Vis absorption spectrum of the synthesized cobalt oxide nanoparticles. The maximum absorption peaks are (230 nm) for *Camellia sinensis* and (225 nm) for *Apium graveolens*, shown in Figure 6. The energy band gaps are (3.6 eV) of camellia sinensi extract and (2.85 eV) of Apium graveolens extract as a result of quantum confinement and small molecules, as show in **Figure 7**.

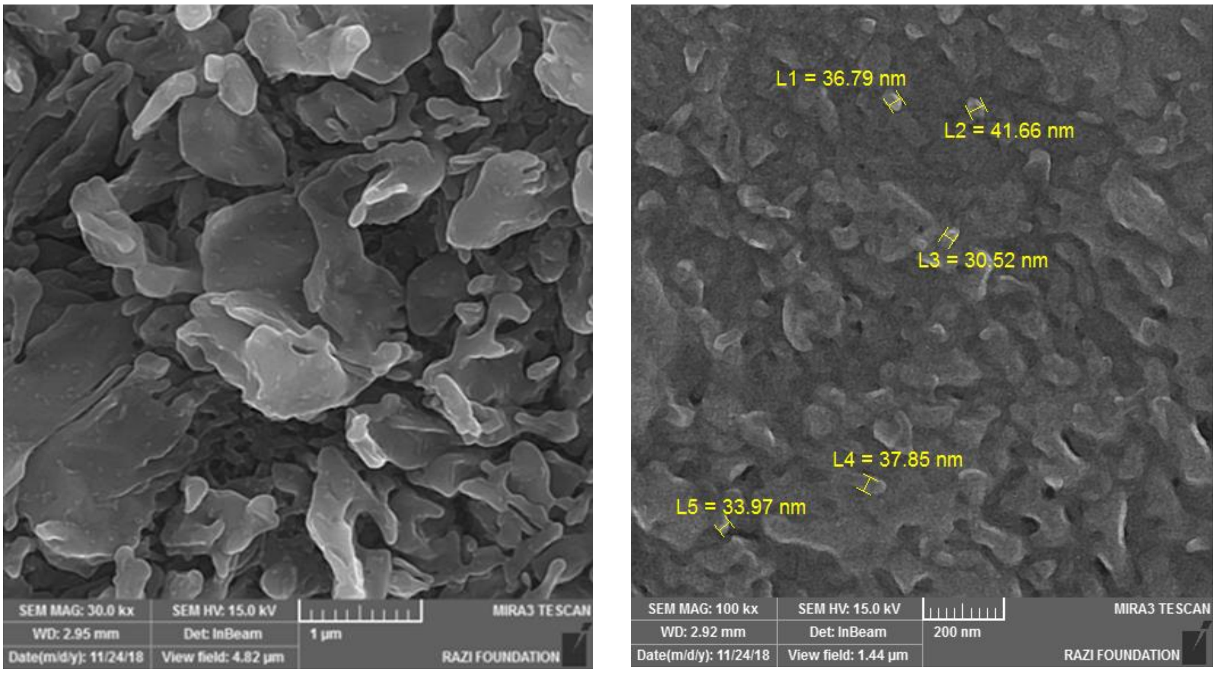

Fig. 4. FE-SEM of cobalt oxide nanoparticles preparing using *Camellia sinensis* extract

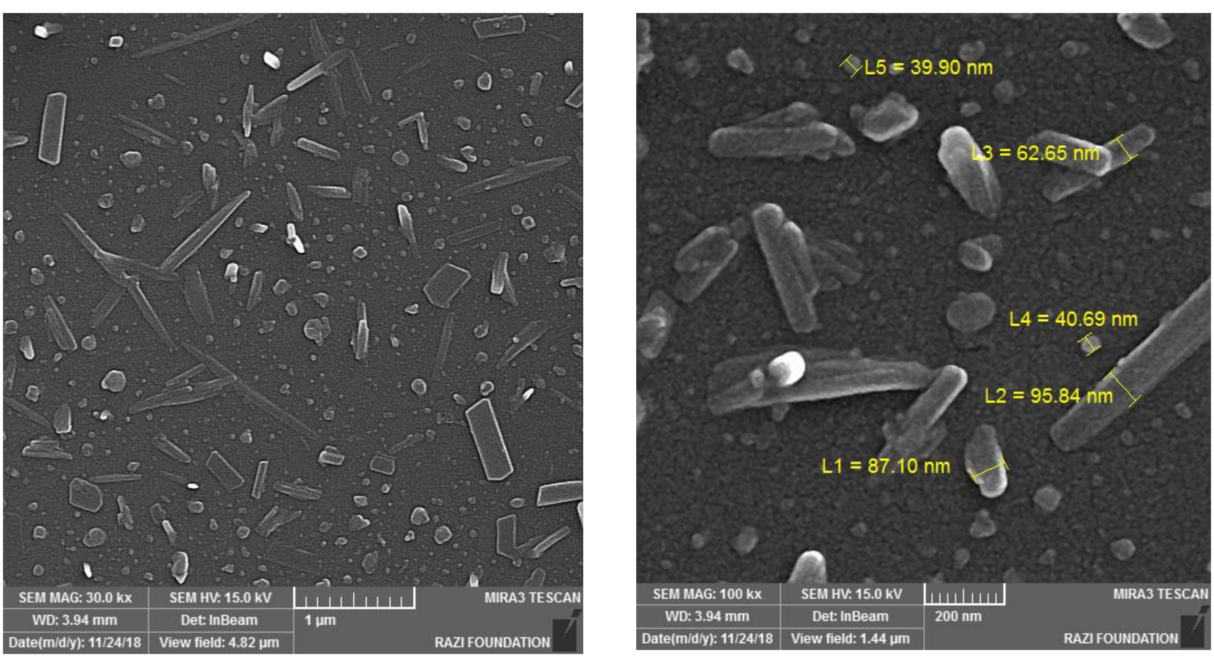

Fig. 5. FE-SEM of cobalt oxide nanoparticles preparing using *Apium graveolens* extract.

3. 4. Antibacterial susceptibility assay

The zone of inhibition of cobalt oxide nanoparticles bio-fabricated from the *Apium graveolens* and *Camellia sinensis* extracts against two pathogens is shown in **Table 1**. Two pathogens, each of Gram-negative (*P. aerugino*) and Gram-positive (*S. aureus*) bacteria organisms were used in this study. These are human pathogens capable of causing diseases ranging from skin infections, pneumonia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, diarrhea, anemia, kidney infections, osteomyelitis, endocarditis, septicemia, lung infection to wound infections. The surfaces of the cobalt oxide nanoparticles might have interacted directly with the bacterial outer membrane, causing the membrane to rupture thereby killing the organism. So, the antibacterial activity exhibited by the cobalt oxide nanoparticles here is attributed to their small size and high surface-to-volume ratio, which allows them to interact closely with microbial membranes.

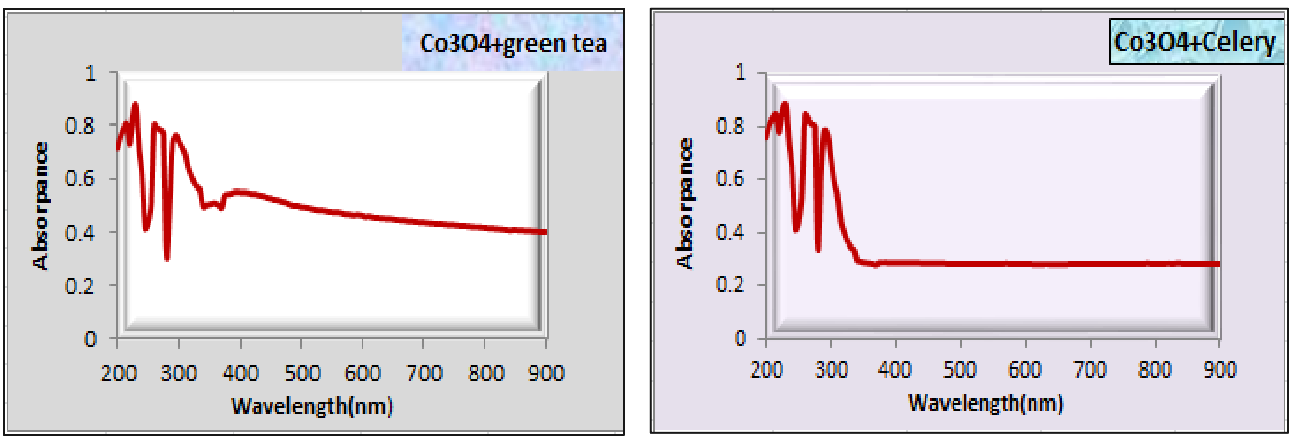

Fig. 6. UV-Vis absorption spectra of cobalt oxide NPs with *Camellia sinensis* extract and *Apium graveolens* extract.

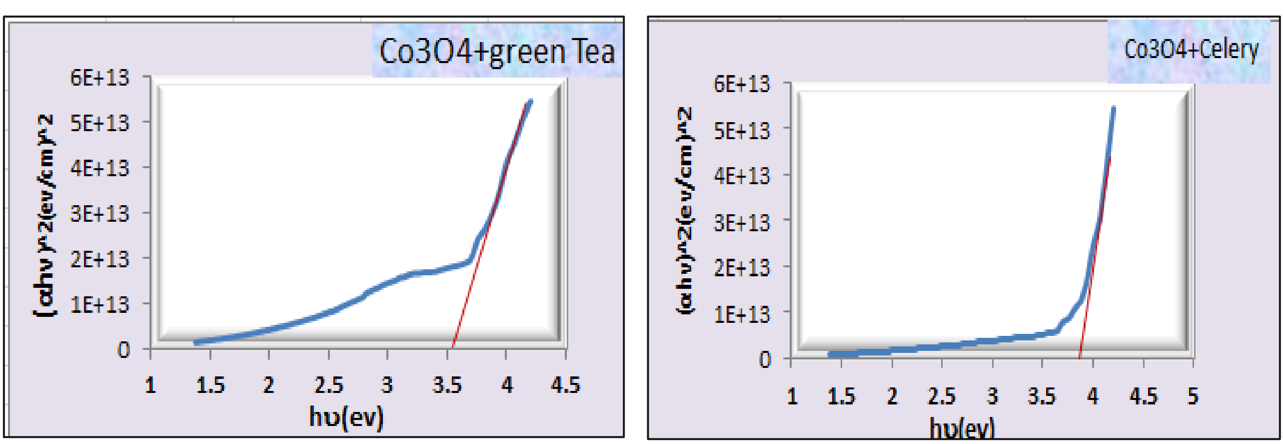

Fig. 7. The energy band gap of cobalt oxide nanoparticles with *Camellia sinensis* extract and *Apium graveolens* extract.

The result was used as a positive control in the experiment. Note that the presence of plant extracts increase the effectiveness of material nanoparticles, as green tea extract increases the proportion of negative bacteria, killing of 25 mm with celery extract and 17 mm without the extract to 29 mm, with leaves extract and the presence of plant extracts increase the effectiveness of oxide nanoparticles, as green tea extract increases the proportion of positive bacteria, killing of 22 mm with celery extract and 15 mm without the extract to 27 mm with leaves extract.

Table 1. Zone Inhibition (mm) of cobalt oxide NPs against pathogens.

Type of material nanoparticles used	Zone of inhibition (mm) at 200 μg/mL concentration *S. aureus*	Zone of inhibition (mm) at 200 μg/mL concentration *P. aerugino*	Percentage of Inhibition (%) *S. aureus*	Percentage of Inhibition (%) *P. aerugino*
Cobalt oxide pure	15	17	13.5	15.3
Cobalt oxide with *Apium graveolens*	22	25	19.8	22.5
Cobalt oxide with *Camellia sinensis* leaves	27	29	24.3	26.1

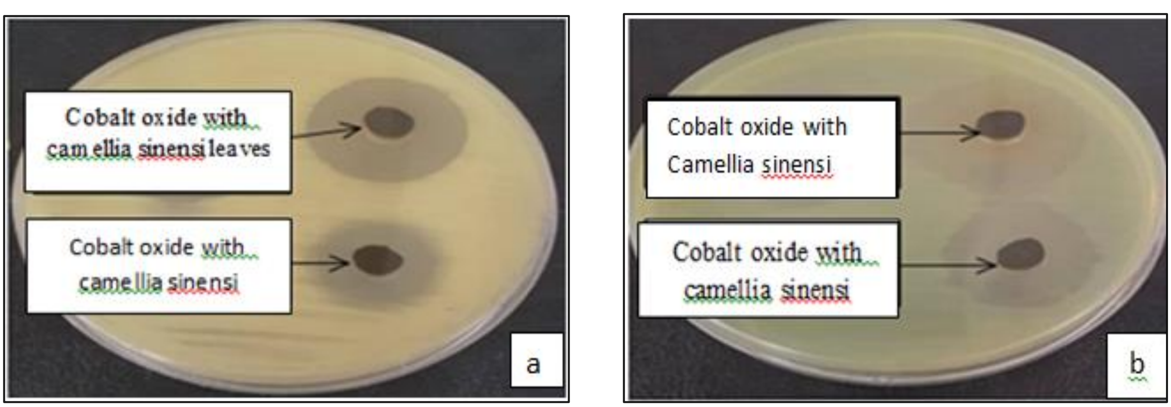

Fig. 8. Rate of inhibition for bacteria *Pseudomonas aeruginosa* a) *S. aureus* b) *P. aeruginosa* for cobalt oxide NPs of plant extracts

4. CONCLUSIONS

Cobalt oxide nanoparticles were synthesized using *Camellia sinensi* leaves and *Apium graveolens* extract, which is a green method of nanoparticles synthesis that. does not introduce harmful substances into the environment and ensures cost effectiveness. The particle size was calculated to be in the range of 21–55 nm. These cobalt oxide nanoparticles inhibited the growth of *S. aureus*, *P. aerugino*. Therefore, it is pertinent to conclude that the cobalt nanoparticles could be used in the treatment of diseases and infections caused by these organisms. The best results were obtained from chemical preparation, simple and clear differentiation peaks of Co_3O_4 NPs with green tea and the best to inhibition the existence of green tea extract was (27-29 mm) of *S. aureus* and *P. aerugino* bacteria, respectively.

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