Antibacterial and Antifungal Efficacy of Phyto-synthesized Cobalt Nanoparticles Synthesized Using Aloe vera (Aloe barbadensis Miller) Extract.

E. Karu [1]
S. Abubakar [1]
M. M. Usman [1]
A. M. Bello [1,2*]

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[1] Gombe State University, Tudun Wada, Gombe State, Nigeria.
[2] Gombe State College of Health Science and Technology, Kaltungo
*Corresponding Author's Email: ahmedmohammedbello70@gmail.com

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Elisha Karu
Gombe State University, Tudun Wada,
Gombe State, Nigeria.

Samaila Abubakar
Gombe State University, Tudun Wada,
Gombe State, Nigeria.

Musbahu Muhammad Usman
Gombe State University, Tudun Wada,
Gombe State, Nigeria.

Ahmed Mohammed Bello
Gombe State University,
Tudun Wada,Gombe State, Nigeria.
Gombe State College of Health Science and Technology,
Kaltungo
ahmedmohammedbello70@gmail.com

Abstract— Antimicrobial resistance is exponentially increasing because microbes evolve new ways to evade treatment. Therefore, threatening our ability to treat common bacterial and fungal diseases. This research work covers the synthesis of cobalt nanoparticles using extract of Aloe vera, characterization, and antimicrobial activity. Phytochemical screening of aqueous extract of Aloe vera was carried out in accordance with established methods. Co NPs were synthesized using the bottom-up method of synthesis and characterized using FTIR, UV-Vis spectrophotometry, SEM, and XRD. Prominent absorption peaks appeared at 3405 cm^{-1}, which was ascribed to OH vibration; 2923 cm^{-1}, assigned to Sp3 C-H; sp^3 1634 cm^{-1}, assigned to C=O; and the characteristic metal-oxygen peak appeared at 650 cm^{-1}. UV-visible analysis showed wavelengths of maximum absorption (λmax) at about 380 nm and 400 nm. The SEM analysis of Co NPs showed irregular morphology. While the closest crystalline structure was body-centered cubic (BCC) with a size of 10.43 nm. The synthesized Co NPs were employed for antibacterial and antifungal studies against two gram-negative bacteria, Escherichia coli and Salmonella typhi. Two gram-positive bacteria, Staphylococcus aureus and Helicobacter pylori, and two fungi, Aspergillus niger and Candida. The result showed the highest inhibition at 28 mm, 26 mm, 20 mm, and 15 mm for E. coli, S. typhi, H. pylori, and Staph. aureus, respectively. Also, 21 and 20 mm for two fungi, Aspergillus Niger and Candida. Augmentin and ketoconazole were used as bacterial and fungal control drugs. The cytotoxicity (brine shrimp lethality assay) of the plant extract did not pose significant acute toxicity to the brine shrimp, while Co NPs demonstrated a clear concentration-dependent pattern.

Keywords— Synthesis, Nanoparticles, Antibacterial, Antifungal

I. INTRODUCTION

Nanotechnology is a field of science that involves the manipulation of matter at the atomic and molecular scales. Nanotechnology has garnered substantial interest due to its wide array of applications in medicine, biology, energy, and industrial technology [1]. Nanoparticles (NPs) are at the forefront of nanotechnology; with at least one dimension less than 100 nanometers, they emerged as key materials in medicine due to their unique properties, such as high surface area to volume ratio, quantum effects, and increased reactivity [2]. Among the various types of metallic nanoparticles, cobalt NPs have drawn significant attention for their potential in a variety of applications, particularly in the biomedical field [3]. Cobalt is a transition metal known for its ferromagnetic properties, making cobalt nanoparticles particularly interesting for applications in magnetic storage devices and magnetic resonance imaging (MRI) contrast agents. The synthesis of cobalt nanoparticles can be achieved through various methods, including chemical reduction, thermal decomposition, and sol-gel processes. Each method offers different advantages in terms of particle size, distribution, and morphology.

In 2019, a study showed that about 3.57 million out of 4.95 million deaths worldwide were associated with antimicrobial resistance, which is higher than other well-known causes of mortality (malaria and HIV/AIDS) [4]. The escalating threat of antimicrobial resistance (AMR) has significantly diminished the efficacy of traditional antibiotics and antifungal drugs against multidrug-resistant (MDR) pathogens, posing a major global health challenge [5]. Resistance arises from complex, multifaceted, and cross-sectoral factors [6]. But the primary driver of resistance is often linked to the misuse of antimicrobials in medicine, veterinary, and agricultural settings [7].[8]. As a result, nanomaterials, particularly metal and metal oxide nanoparticles, have emerged as promising alternatives due to their distinctive properties, such as high surface area-to-volume ratios and the ability to generate reactive oxygen species (ROS) [9].[10]. Cobalt nanoparticles have been shown to inhibit the growth of various bacterial strains, including both gram-positive and gram-negative bacteria [11]. The mechanism of action often involves the disruption of bacterial cell membranes, leading to cell lysis and death. Furthermore. The antimicrobial efficacy of cobalt nanoparticles is influenced by several factors, including particle size and large surface area, which enhance ROS production and disrupt fungal cell structure [12]. Smaller nanoparticles often exhibit enhanced antimicrobial activity due to their increased surface area and greater ability to interact with microbial cells. Surface modifications with specific ligands or coatings can further improve their antimicrobial properties by enhancing their cellular uptake and selectivity [1]. “However, the exact mechanisms of the antibacterial effect of nanometals are still one of the most researched topics” [13].

The possible toxic effects of nanoparticles include production of inflammatory mediators, damage to cell membranes, damage to DNA, and altering cellular redox balance through oxidative stress, which leads to abnormal functioning of cells or cell lysis [14]. Despite the promising applications of cobalt nanoparticles, there are concerns regarding their toxicity and environmental impact. Studies have indicated that cobalt nanoparticles can induce oxidative stress and cytotoxicity in various cell types, raising questions about their safety for biomedical applications. But [15] argued that Co NPs had no adverse effect and can be employed for biomedical applications.
Aloe vera (*Aloe barbadensis Miller*) is a widely known medicinal plant that has shown great promise as a reducing and stabilizing agent in nanoparticle synthesis [16]. Aloe vera contains a range of bioactive compounds, including flavonoids, polyphenols, saponins, and other phytochemicals, which have demonstrated the ability to reduce metal salts into nanoparticles [17]. Due to its natural constituents, aloe vera is an ideal candidate for the green synthesis of cobalt nanoparticles.

II. METHODOLOGY

A. Sample Collection

Matured aloe vera leaves were collected within Gombe metropolis, Gombe State, Nigeria. The plant leaves were identified and authenticated by the Gombe State University herbarium section of the Department of Biological Sciences of the University. They were washed four times with tap water, rinsed with distilled water to remove adhered dirt, and then air-dried for one week. They were ground using a wooden mortar and pestle and sieved into fine powder.

B. Extract Preparation

About 30 g of the powdered sample of *aloe vera leaves* were weighed and dispersed into a 500 cm³ beaker to which 200 cm³ of distilled water were added and boiled at 80°C for 30 minutes. The mixture was allowed to cool and filtered using Whatman No. 1 filter paper. The filtrate was then centrifuged at 250 rpm for 2 hours; it was then retained for the synthesis of Co NPs.

C. Determination Of Phytochemical

Aloe vera leaf extract was subjected to preliminary phytochemical screening for detection of the following constituents as reported by [18].
Saponins: About 1 cm³ of the extract was added to 2 cm³ of distilled water in a 100 cm³ beaker and shaken vigorously. The formation of bubbles was taken as a positive test for saponin constituents.
Flavonoids: About 3 cm³ of Aloe vera extract was taken into a test tube and treated with 1 cm³ of 10% NaOH solution. The formation of an intense yellow color was observed that disappeared after the addition of a few drops of 2M H_2SO_4, confirming the presence of flavonoids.
Phenols and Tannins: Approximately 10 cm³ of the extract was taken in a test tube to which 0.5 cm³ of 1% lead acetate solution was added from a 1 cm³ graduated pipette. A dark brown precipitate was formed, indicating the presence of tannins and phenol.
Alkaloid: About 1 cm³ of Mayer's reagent was added dropwise to 10 cm³ of the extract. The presence of a greenish color or cream precipitate indicates the presence of an alkaloid.
Glycoside: Approximately 1 cm³ of the extract 1.5 cm³ glacial acetic acid was added, then 1 drop of 5% ferric chloride solution, followed by the addition of concentrated sulphuric acid along the side of the test tube. The appearance of blue color indicates the presence of glycoside.
Terpenoid: Approximately 2 cm³ of the extract was treated with 2 cm³ of acetic acid, then concentrated sulphuric acid was added dropwise. Deep red color development showed the presence of terpenoids.

D. Synthesis of Cobalt Nanoparticles (Co NPs)

The method of [19] was adopted for the synthesis of Co NPs. The prepared $CoSO_4.7H_2O$ (0.01 mol) solution was added dropwise into the plant extract in a ratio of 1:5 with constant stirring at 80°C for 60 minutes using a glass rod stirrer. Within the first 15 minutes the color changed from yellow to red, which indicated the formation of Co NPs nanoparticles. The Co NPs were then allowed to settle for 24 hours (one day), after which they were decanted and dried at 100°C for 4 hours and then ground into powder for further analysis.

E. Characterization

The synthesized NPs were characterized using the following techniques:

F. UV-Visible Spectroscopy

UV-visible (Perkin Elmer spectrophotometer model 725) was used to observe the wavelength of maximum absorbance and attribute it to surface plasmon resonance. A clean cuvette was filled with distilled water and used as a blank. Another cuvette was filled with the aliquot of Co NPs and scanned from 200 nm to 800 nm.

G. Scanning Electron Microscopy (SEM) Coupled with Energy Dispersion X-ray (EDX) Analysis

The SEM coupled with EDX analysis was carried out at the Centre for Energy, Research and Training (CERT), Zaria, Kaduna State. Nigeria in order to determine the morphology, size, and composition of the elements present in the synthesized manganese and cobalt nanoparticles.

H. Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was conducted on both the plant extract and cobalt nanoparticles to determine the functional groups responsible for the reduction, capping, and stabilization of the Co NPs. A spectrophotometer model 10.03.09 at the Pharmaceutical Department, Gombe State University, was used. The IR spectra were acquired by the KBr pellet technique.

I. X-Ray Diffraction (XRD) Analysis (X-Pert Plus)

XRD analysis was carried out at the Centre for Energy, Research and Training (CERT), Zaria, Kaduna State. Nigeria in order to find out the average crystalline size using Cu-Kα radiation in a 0-2θ configuration with a wavelength (λ) of 1.5411Å. The Debye-Scherer equation was used to calculate the average crystalline size.
The Debye Scherer equation is as follows

$$D = K\lambda/\beta COS\theta$$

Where D= Particles size = 0.94
K= Constant volume
λ= X-ray wavelength(0.154nm)
P=Line broadening at half the maximum intensity
θ= Braggs angle (in degree).

J. Media Preparation

Approximately 38 g of nutrient agar was dissolved into 1000 cm³ of deionized water and autoclaved for 15 minutes at 120°C to sterilize.

K. Brine Shrimp Hatching

The brine shrimp hatching was carried out using the method of [20]. About 27 g of NaCl (table salt) was weighed and transferred into a jar containing 1000 cm³ of water. The solution was stirred with a spatula, and the tip of an airline from an air pump was placed into the bottom of the jar to enable proper aeration. 1 g of brine shrimp eggs was added at the top water level of the jar and mixed. A light source (60–100-watt bulb) was placed a few inches away from the jar. After 20-24 hours, the nauplii hatched. The nauplii were collected after 24 hours, and 10 of the nauplii were transferred to a beaker using a Pasteur pipette.

L. Bioactivity Studies

The antimicrobial activity studies were carried out by using the agar well method to test the antimicrobial activity of the green synthesized manganese and cobalt nanoparticles on two gram-negative bacteria, Escherichia coli and *Salmonella typhi*. Two gram-positive bacteria, Staphylococcus aureus and *Helicobacter pylori*, and two fungi, *Aspergillus niger and Candida albicans*. This was conducted by creating a 6 mm hole in the prepared agar (media) inside the petri dish. The organism was inoculated all over the surface of the petri dish, and the synthesized nanoparticles (500 µg/cm³, 250 µg/cm³, 125 µg/cm³, and 62.5 µg/cm³) were also inoculated into each hole with a control drug at the center. It was then incubated overnight at 37°C, after which the zone of bacteria and fungi growth inhibition was measured in millimeters (mm). Minimum inhibition concentration (MIC) was also evaluated.

M. Cytotoxicity (Brine Shrimp Lethality) Assay

The cytotoxicity of aloe vera plant extract was carried out using a brine shrimp lethality assay to check the cytotoxic effect of the bioactive chemicals or efficacy of phytochemicals present in the sample. The lethality concentrations of the extract and the Co NPs were 100 µg/cm³, 50 µg/cm³, and 25 µg/cm³..

III. RESULT AND DISCUSSION

Table 1: Results of the phytochemical analysis of the aloe vera leaf extract

1	Alkaloids	++
2	Flavonoids	+
3	Saponins	++
4	Phenols and tannins	+
5	Glycosides	+
6	Terpenoid	-

Keyword: + = detected, ++ = much detected, - = not dectected

Six phytochemicals were screened; out of the six, five (5) were found to be present, while terpenoid was not detected. But alkaloids and saponins were found to be in appreciable quantity. The phytochemicals detected are in line with the functional groups confirmed by the IR analysis. This finding aligns with that of [18][21].

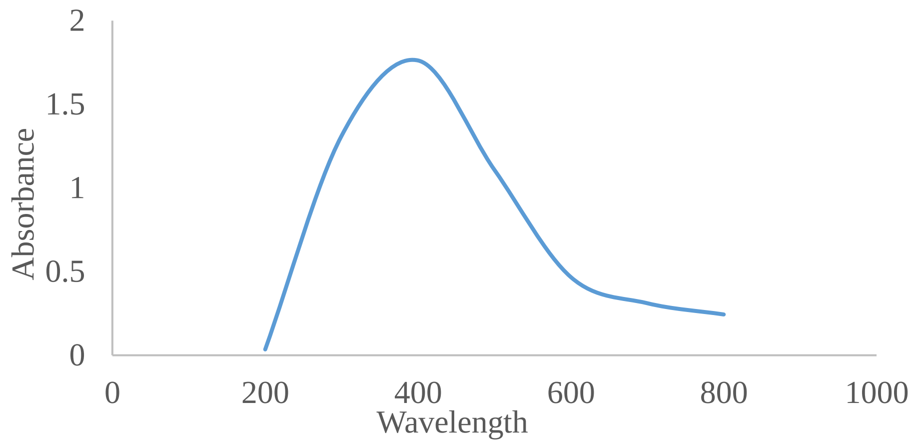

Figure 1: Result of UV-vis Analysis of Co NPs

The formation of cobalt nanoparticles was confirmed by UV–vis spectroscopy. Which showed peaks with maximum absorbances between 380 nm and 400 nm, generally ascribed to the Surface Plasmon Resonance (SPR) phenomenon as reported by [19]. This result is similar to that of [22].

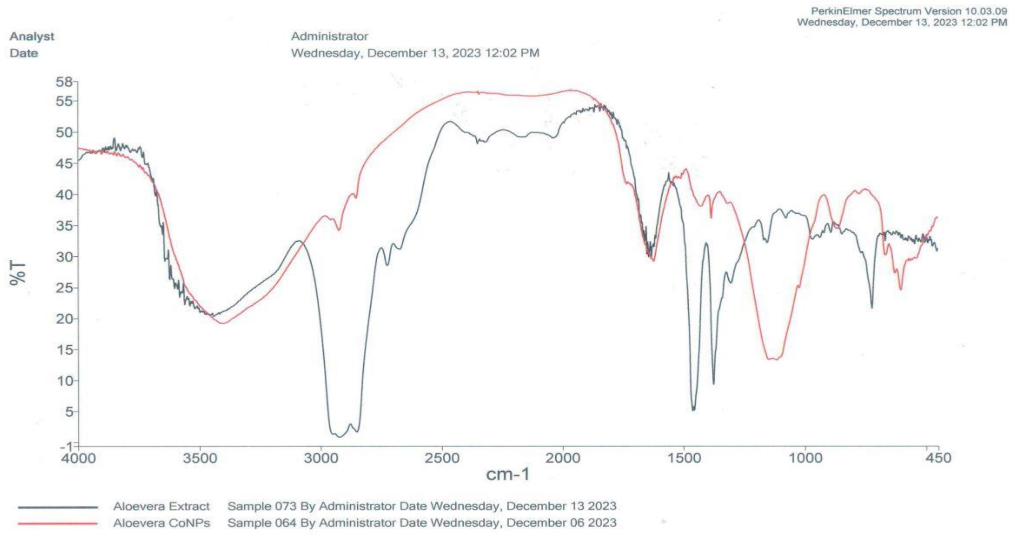

Figure 2: Superimposed FTIR result of Aloe vera extract and Co NPs

Major peaks observed are broad peaks at around 3605 cm⁻¹ and 3405 cm⁻¹ in both the aloe vera extract and the Co NPs, respectively. Another peak around 2923 cm⁻¹ in both the aloe vera extract and the cobalt NPs. Small peaks were observed at 2725 cm⁻¹ and 2672 cm⁻¹ in the IR spectra of the extract and the nanoparticles. Medium peaks occurred at 1659 cm⁻¹ and 1622 cm⁻¹ for plant extract and Co NPs, respectively. This assignment is in fairly good agreement with the assignment of [19][15]. And, produced good evidence of the phytochemicals such as phenolic groups, flavonoids, and tannins acting as reducing, stabilizing, and capping agents for the synthesis of nanoparticles. A bending vibration was observed at 650 cm⁻¹ for cobalt nanoparticles that was totally absent from that of the plant extract and was assigned to Co-O.

Table 2: Absorption frequencies and assignment of superimposed FTIR result of Aloe vera and Co NPs

Aloe vera Extract (cm1)	Co NPs (cm1)	Assignment
3605br	3405br	vOH stretching vibration probably from phytochemicals such as phenol, carboxylic acid, alcohol and water.
2923m	2923m	vC-H stretching from alkane
2725w	N.O	vC-H stretching due to H-bonded alkenes
2672w	N.O	
1622w	1634w	vC=O stretching vibration due carbonyl functional group from carboxylic acid and flavanoid or tannins.
1376w	1384w	C-H bend alkanes
N.O	650w	VCo-O Nps formation
1076w	1050w	vC-N

Note: vs = strong, v = strong, m = medium, w = weak, br = broad, sh = shoulder, N.O = not observe

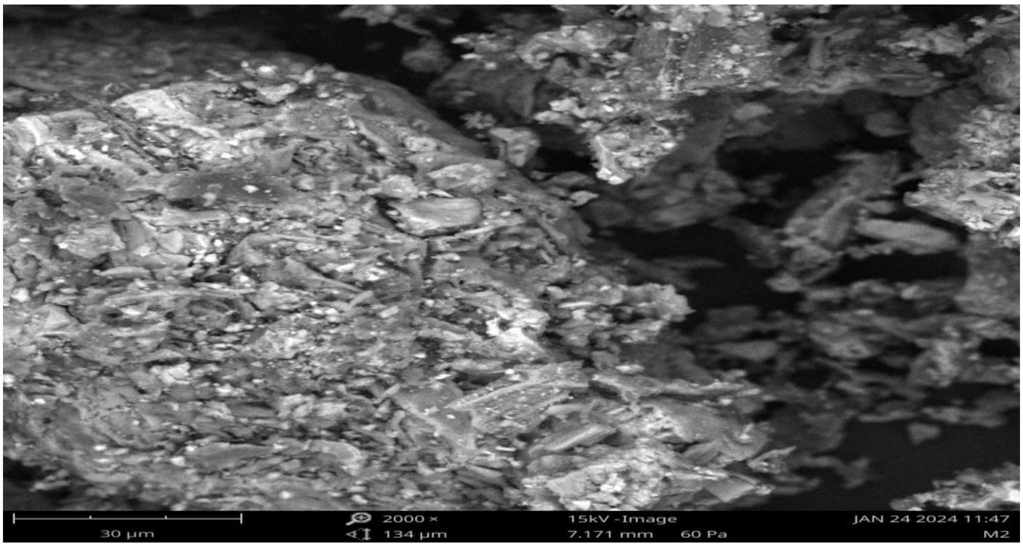

Figure 3: SEM image of the synthesized Co NPs

It is clear from the image obtained from scanning electron microscopy (SEM) that the image at 2000 magnification shows that particles have irregular morphology and are mostly agglomerated and crystalline in nature. This finding is similar to that reported by [23].

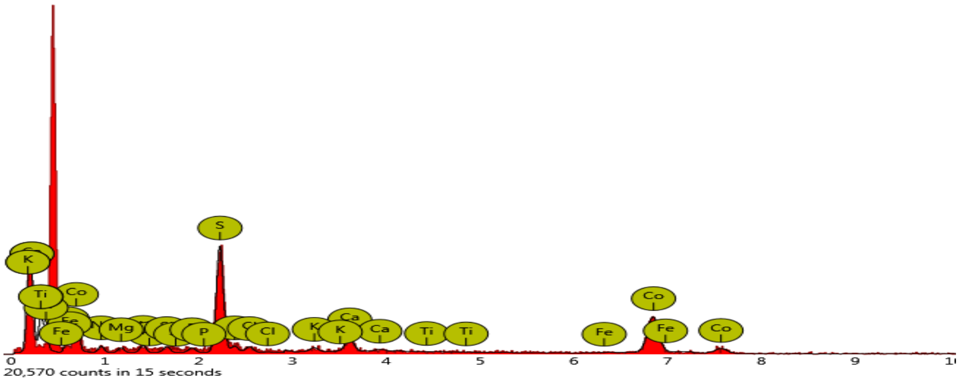

Figure 4: present the result of EDX analysis of Co NPs

The energy dispersion X-ray (EDX) analysis of Co NPs showed various elemental compositions, as shown in Fig. 4. These elements include cobalt (53.99%), sulfur (25.63%), calcium (5.51%), sodium (4.18%), chlorine (2.03%), aluminum (1.96%), and magnesium (1.96%). Other elements are in trace quantities, probably due to their presence in the metal precursor.

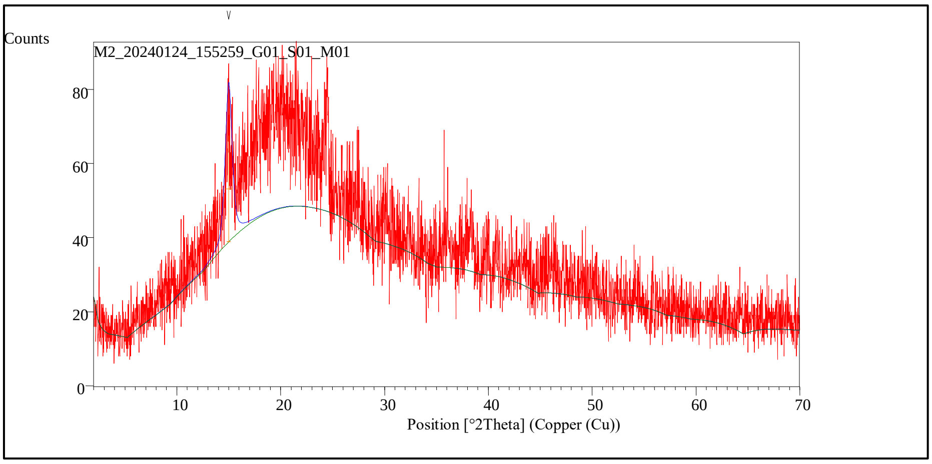

Figure 5: XRD result of synthesized Co NPs

The result showed only one most prominent peak observed at a Bragg angle of 2ϴ 14.980, a peak intensity of 28.74, an interplanar spacing (Å) of 5.90084, and an FWHM left of 0.7680 with respect to a KHL value of (202). The result obtained showed that the closest structure that conforms with the aforementioned parameters is a body-centered cubic (BCC) structure with the average crystalline or particle-edged length of 10.43 nm as calculated by the Scherer equation. The crystalline structure cannot be ascertained due to the large interplanar spacing of 5.90084. This finding is similar to the work reported by [24].

Table 3: Results of antimicrobial and Antifungal activity of aloe vera plant extract

S/N	**Organisms**	**Concentrations**				**Positive Control**
		500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	300µg/ml
1.	E. coli	9mm	8mm	7mm	6mm	35mm
2.	Sal. typhi	8mm	7mm	7mm	7mm	25mm
3.	H. pylori	11mm	8mm	8mm	8mm	20mm
4.	Staph. Aureus	8mm	7mm	7mm	7mm	20mm
						500µg/ml
5.	Candida	9mm	8mm	8mm	7mm	13mm
6.	A. niger	11mm	8mm	8mm	8mm	21mm

Keyword: mm = millimeter, E. coli = Escherichia coli, Staph. aureus = Staphylococcus aureus, A. niger = Aspergillus niger, Candida = Candida albicans, Sal. typhi = Salmonella typhi and H. Pylori = Helicobacter pylori

From table 3 it can be seen that the antibacterial activity of the aloe vera extract showed low growth inhibition against two gram-negative bacteria (E. coli and Sal. typhi), two gram-positive bacteria (Staph. aureus and H. pylori), and two fungi (A. niger and Candida). In comparison, the control drugs (Augmentin and ketoconazole) showed significantly higher inhibition zones.

Table 4: Results of minimum inhibitory concentration (MIC) of Aloe vera extract

S/N	**Organisms**	**Concentrations**				
		62.5µg/ml	31µg/ml	15.5µg/ml	7.5µg/ml	3.5µg/ml
1.	E.coli	-	-	+	+	+
2.	Sal. Typhi	-	+	+	+	+
3.	H. pylori	-	-	+	+	+
4.	Staph. Aureus	-	-	+	+	+
5.	Candida	-	-	+	+	+
6.	A. niger	-	+	+	+	+

+ No turbidity/inhibition, - turbidity/growth

From the table it can be seen that only Sal. typhi and A. niger showed inhibition at 31 µg/ml. The minimum inhibition growth of aloe vera is significantly higher than that of Augmentin and ketoconazole, indicating lower potency.

Table 5: Antimicrobial and Antifungal activity of Co NPs

Organisms	**Concentrations**				**Positive Control**
	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	300 µg/ml
E.coli	28mm	20mm	17mm	14mm	26mm
Sal. Typhi	26mm	17mm	17mm	14mm	18mm
H. pylori	20mm	16mm	12mm	8mm	20mm
Staph. Aureus	15mm	10mm	7mm	6mm	24mm
					500µg/ml
Candida	21mm	20mm	14mm	13mm	20mm
A. niger	20mm	17mm	10mm	9mm	22mm

Keyword: mm = millimeter, E. coli = Escherichia coli, Staph. aureus = Staphylococcus aureus, A. niger = Aspergillus niger, Candida = Candida albicans, Sal. typhi = Salmonella typhi and H. Pylori = Helicobacter pylori

The antibacterial activity test of Co NPs demonstrated increased inhibition growth for E. coli, Sal. Typhi, and Staph aureus (28 mm, 26 mm, and 20 mm) at 500 µg/ml, which is higher than the control Augmentin (26 mm, 18 mm, and 20 mm), respectively. While the inhibition growth of Staph aureus (15 mm) is less than that of the control (24 mm). Also, the antifungal activity of Co NPs against Candida (21 mm) achieved inhibition zones that are greater than those of the control drug ketoconazole (20 mm). While the inhibition zone of A. niger (20 mm) at 500 µg/ml is less than that of the control. This finding partially aligns with the report of [25][11].

Table 6: Result of minimum inhibitory concentration (MIC) of Co NPs

S/N	**Organisms**	**Concentrations**				
		62.5µg/ml	31µg/ml	15.5µg/ml	7.5µg/ml	3.5µg/ml
1.	E.coli	-	-	-	-	+
2.	Sal. Typhi	-	-	-	+	+
3.	H. pylori	-	-	-	-	+
4.	Staph. Aureus	-	-	-	+	+
5.	Candida	-	-	+	+	+
6.	A. niger	-	-	-	-	+

+No turbidity/inhibition, -turbidity/growth

The result of MIC of Co NPs as shown in the table indicated broad-spectrum activity inhibiting all the tested organisms at a concentration ranging from 7.5 µg/ml to 3.5 µg/ml, with the exception of Candida, which is inhibited at 15.5 µg/ml. This suggests potential as an effective antimicrobial agent across both bacterial and fungal pathogens. Although the results showed lower potency than Augmentin and Ketoconazole, it is quite promising; therefore, further optimization is needed to unveil its true potential.

Table 7: Results of cytotoxicity of Aloe vera extract

S/N	**Concentration (µg/ml)**	**No. of live nauplii**	**No. of live nauplii**	**No. of live nauplii**
		Day 1	Day 2	Day 3
1	100	10	10	10
2	50	10	10	10
3	25	10	10	10

The cytotoxicity test result for aloe vera extract at concentrations of 100 µg/ml, 50 µg/ml, and 25 µg/ml, Artemia salina nauplii exhibited complete survival throughout the 3-day exposure period, with no observable mortality. The nauplii demonstrated 100% viability on Days 1, 2, and 3, indicating that, under the experimental conditions, the aloe vera extract did not induce cytotoxic effects at the concentrations assessed. This result is in agreement with the result reported by [26].

Table 8: Cytotoxicity of the synthesized Co NPs

S/N	**Concentration (µg/ml)**	**No. of live nauplii**	**No. of live nauplii**	**No. of live nauplii**
		Day 1	Day 2	Day 3
1	100	10	6	3
2	50	10	8	6
3	25	10	7	5

The cytotoxicity test result for Co NPs, at the highest concentration of 100 µg/ml, showed a noticeable decline in nauplii survival, with 60% survival recorded on Day 2 and 30% on Day 3, indicating a significant toxic effect that intensified over time. At 50 µg/ml, survival rates were more stable, with 80% survival on Day 2 and 60% on Day 3, suggesting that the nanoparticles' toxicity was lower than at the highest concentration but still evident. At the lowest concentration (25 µg/ml), mortality was least pronounced, with 70% survival on Day 2 and 50% on Day 3, highlighting a less severe but still notable toxic effect. These results demonstrate a clear concentration-dependent pattern in nanoparticle toxicity. This finding is in agreement with the result reported by [20].

IV. CONCLUSION

The phytochemicals present in Aloe vera include alkaloid, flavonoid, saponin, phenols and tannins, and glycoside, while terpenoid was not detected. Co NPs was successfully synthesized using extract of Aloe vera and subjected to a series of characterization techniques: UV-vis, FTIR, SEM, and XRD. The synthesized cobalt nanoparticles were found to have a basic centered cubic (BCC) crystalline shape with a 10.43 nm size.

The aloe vera extract showed no significant antimicrobial activity compared to the control. While cobalt nanoparticles, on the other hand, show excellent antimicrobial activity against two gram-negative bacteria (E. coli and Sal. typhi), two gram-positive bacteria (Staph. aureus and H. pylori), and two fungi (A. niger and Candida). The cytotoxicity is less severe but concentration dependent. Therefore, Co NPs can be used or incorporated in antibiotics and antifungal drugs.

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