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Green Synthesis of Silver Oxide Nanoparticles using Zingiber officinale Extract: Catalytic and Antibacterial Potentials of 2-Phenyl Benzimidazole Derivatives

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ABSTRACT: This study presents a novel and environmentally friendly method for synthesizing silver oxide nanoparticles (Ag_2O NPs) using Zingiber officinale root extract, reducing the traditional synthesis method's environmental impact. The synthesized nanoparticles were highly effective catalysts for synthesizing 2-phenyl benzimidazole (BI) derivatives under mild conditions, with excellent yields ($83-92\%$). The synthesized NPs were characterized by FTIR, SEM/EDX, and XRD. The characterization of the prepared compounds provides valuable insights into their chemical composition, enabling further optimization of their properties. The Ag_2O nanoparticles catalyzed BI derivatives were tested for antibacterial activity against Escherichia coli, showing a maximum zone of inhibition of 25 ± 0.23 mm. The significance of this work lies in the potential applications of these derivatives in medicine, materials science, and agriculture. Overall, the green and sustainable approach in this study to nanoparticle synthesis has significant implications for the development of new, eco-friendly technologies, making it of significant scientific interest.

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I. INTRODUCTION

Heterocyclic compounds play an essential role in various fields of life sciences, serving a wide range of significant functions in nature, medicine, and innovation. The nitrogencontaining azoles family is of particular interest in synthesis due to its prevalence in pharmacologically active natural products, and these molecules are useful in pharmaceuticals, agrochemicals, and other materials[1]. Benzimidazoles are a versatile class of natural and synthetic heterocyclic compounds with important biological and pharmaceutical properties, including antihistaminic[2], antitumor[3], antiparasitic[4], antiarrhythmic[5], antiviral[6] and antiulcer[7] effects. Derivatives of benzimidazole have also been found to have biological activity as fungicides, herbicides, and materials with excellent properties in dyes and high-temperature polymers[8]. Benzimidazole is a naturally occurring structural nucleotide and is also an essential factor in vitamin B[9]. Additionally, benzimidazole derivatives are commonly used in the production of commercial medicines such as proton pump inhibitors Thiabendazole and Omeprazole.

In literature, the most favorable method for synthesis of 1,2-disubstituted benzimidazoles is direct condensation-aromatization, i.e.; the reaction of *o*-Phenylenediamine (o-PDA) with aldehyde[10]. Multiple reactions have also been carried out with various metal catalysts including $Yb(OTf)_3$[11], $CeCl_3 7H_2O/NaI$[12], SmI_2[13], $YbCl_3$[13], $HClO_4$ on silica gel[14], ionic liquid[15] and microwave conditions[16]. Although numerous published procedures for the synthesis of benzimidazole derivatives have been successful, some have exhibited disadvantages such as poor yields, various side reactions,

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prolonged reaction times, drastic reaction conditions, high reaction temperatures, and the use of high-cost reagents and toxic solvents. As a result, many scientists and researchers prefer a mild and practical approach to the synthesis of benzimidazole derivatives[17].

In the present era of nanotechnology, the focus is on synthesizing oxide nano-structures that possess interesting and useful properties compared to their larger counterparts. At the nanoscale level, metal oxide materials can exhibit unique physical and chemical characteristics such as high-surface chemistry, surface area, and high density. Nanocatalysts, which are typically composed of nanoparticles (NPs), have a significant impact on various synthesized products in both organic and inorganic synthesis[18] and many metal oxide catalysts developed as heterogeneous catalysts for the synthesis of benzimidazole derivatives[19]. Silver oxide NPs possess various applications such as pharmaceutical products, antimicrobial agents, textiles, construction materials, the food industry, electronics, cosmetics, and paints. In recent research studies, silver oxide NPs considered an effective topic due to their cost-effectiveness and eco-friendliness. The shape and size of Ag_2O NPs produced by plant sources are the most stable and relatively popular[20].

There are also numerous biological applications of silver oxide NPs such as antifungal, antibacterial, antiviral, therapeutic, and cancer treatment due to their small size and diameter[21]. Ag_2O nano-catalyst has a broad range of applications[22], and the study of green synthesis of Ag_2O NPs remained an attraction for many scientists and researchers for being cost-effective and non-toxic[23]. The nanocatalysts prepared by plant extract are the most stable and renowned due to their size and shape. Different parts of the plant including stem, seed, latex, root, and leaf are being used for the synthesis of silver oxide (Ag_2O) nanocatalyst[24,25]. The choice of Zingiber officinale for the green synthesis of Ag_2O nanoparticles in this study was influenced by its widespread availability and its rich composition of bioactive phytochemicals, including terpenoids, flavonoids, and phenolic compounds. Previous studies [26-28] have shown that it is effective as both a reducing and stabilizing agent in nanoparticle synthesis. Additionally, Zingiber officinale has demonstrated antibacterial and antioxidant properties in various biological activities.

This study presents a simple and effective method for synthesizing 2-phenyl benzimidazole derivatives using o-phenylenediamine (o-PDA) and aldehydes or carboxylic acids in toluene solvent under ambient conditions, utilizing silver oxide nanocatalyst (green synthesis approach) and Zingiber officinale extract for the evaluation of catalytic activity in the reaction as well as its antibacterial activities. This study specifically aims to:

- Develop a green synthesis protocol for silver oxide (Ag_2O) nanoparticles (NPs) using Zingiber officinale extract.
- Characterize the prepared Ag_2O NPs.
- Synthesize and characterize a series of 2-phenyl benzimidazole derivatives in the presence and absence of Ag_2O NPs as a catalyst.
- Evaluate the catalytic activity of Ag_2O NPs in the preparation of benzimidazole derivatives.
- Assess the antibacterial efficacy of the synthesized compounds against Escherichia coli.

To the best of the authors' knowledge, this approach has not been reported in previous studies. Therefore, achieving the aforementioned objectives will contribute to the development of sustainable and effective catalytic and antibacterial agents, potentially addressing the increasing challenges in synthetic chemistry and antibiotic resistance.

II. MATERIALS AND METHODS

A. MATERIALS

The Zingiber officinale (ginger) root used in this experiment was fresh and purchased from the local market, near the University of Lahore, Sargodha. Silver Nitrate ($AgNO_3$; 99.9%), ortho-phenylenediamine (OPDA; 99.5%), benzaldehyde ($C_7H_6O; \geq 99.5\%$), and benzoic acid ($C_7H_6O_2; \geq 99.5\%$), were purchased from Sigma Aldrich. The analytical-grade ethanol ($C_2H_5OH; \geq 99.45\%$) and toluene ($C_6H_5CH_3$; 99.8%) solvents were purchased from Sigma Aldrich and used without purification.

B. CHARACTERIZATION

The FTIR technique was utilized to identify the functional groups present in both the synthesized silver oxide nanoparticles and benzimidazole derivatives. A Perkin Elmer / spectrum II instrument was used to collect the FTIR-ATRA spectra in the transmission mode, ranging from 8550 to 4000 cm − 1. X-ray diffraction (XRD) was conducted using a PANalytical $\theta - 2\theta$ instrument to study the samples. The 1 H NMR and 13 C NMR spectra were obtained using a spectrophotometer with frequencies of 400 MHz and 100 MHz, respectively, to confirm the structures of the compounds. The samples were dissolved in deuterated chloroform as solvent and tetramethylsilane (TMS) was used as an internal reference, using a Bruker Avance III HD 400 MHz instru-

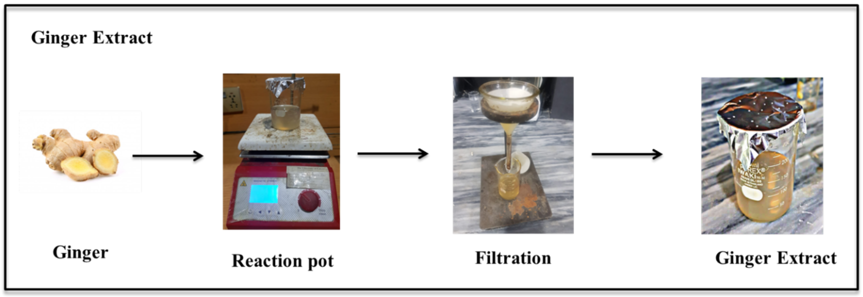


FIG. 1: Preparation of *Zingiber officinale* extract.

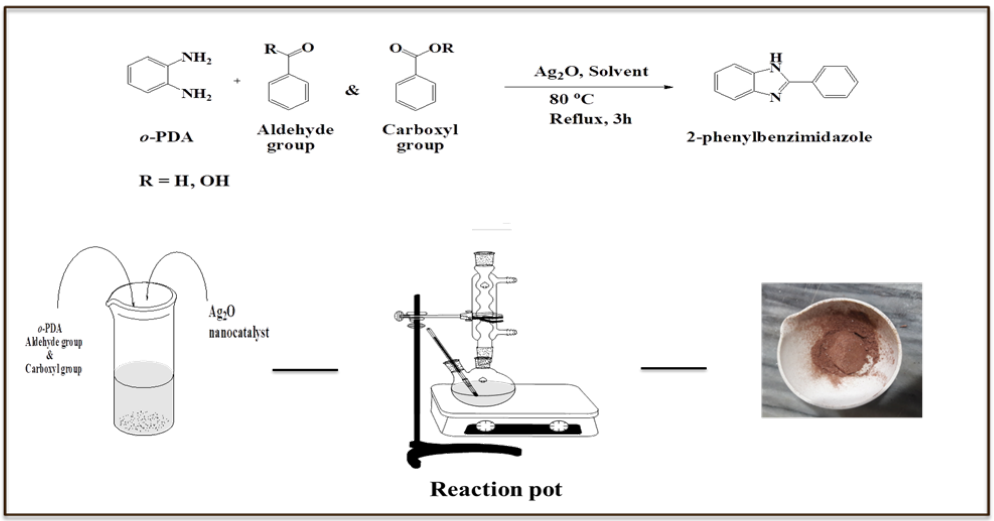


FIG. 2: Scheme for the synthesis of 2-phenylbenzimidazole.

ment. Scanning electron microscopy (SEM) was employed to examine the morphology of the silver oxide nanoparticles. Thin layer chromatography (TLC) was performed using aluminum sheets coated with silica gel 60 (MERCK) containing fluorescent indicators, and the solvent used for development of the TLC plate was a mixture of hexane and ethyl acetate (7:3).

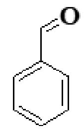
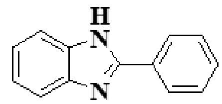
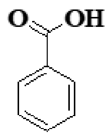
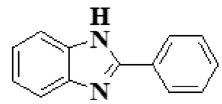
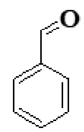
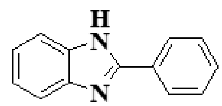
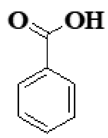
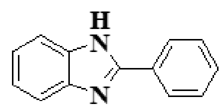
C. Preparation of Zingiber officinale (Ginger) Extract

The extract of Zingiber officinale (ginger) was prepared by taking 20 grams of small ginger pieces and boiling them in 250 mL of distilled water for 30 minutes. After heating, the extract was allowed to cool to room temperature and then filtered using Whatman filter paper to remove any suspended impurities. A schematic representation of the extract preparation process is shown in Fig. 1.

D. PREPARATION OF THE SILVER OXIDE NANOPARTICLES (AG 2_2O NPS)

First, 0.5 g silver nitrate ($AgNO_3$) was dissolved in 90 mL of distilled water and then 30 ml of prepared ginger extract was added to it dropwise. The mixture was heated for 25 to 35 minutes under continuous magnetic stirring. The formation of dark brown precipitates indicated the presence of silver oxide nanocatalyst. After cooling, the mixture was centrifuged at 35000 rmp for 20 minutes to collect the silver oxide precipitates. Finally, the obtained nanoparticles were dried in the oven at 90°C for 3 to 4 hours. The dried silver oxide nanoparticles were black and stored for further use. The synthesis of silver oxide nanoparticles from ginger extract has proven to be more effective than other plant-based sources such as aloe vera, neem, and tea extract, owing to its high phytochemical stability, ease of extraction, and enhanced yield of nanoparticles[26,27].

Table 1: Summary of reaction conditions for the synthesis of BZI derivative with & without Ag_2O nanocatalyst

Entry no.	Substrate	Ag_2O Catalyst	Temp.	Solvent	Time (h)	Product	Yield
AT-2		Yes	Reflux 80 °C	Toluene	3		89 %
CT-2		Yes	Reflux 80 °C	Toluene	3		92 %
AT-1		No	Reflux 80 °C	Toluene	3		85 %
CT-1		No	Reflux 80 °C	Ethanol	3		83 %

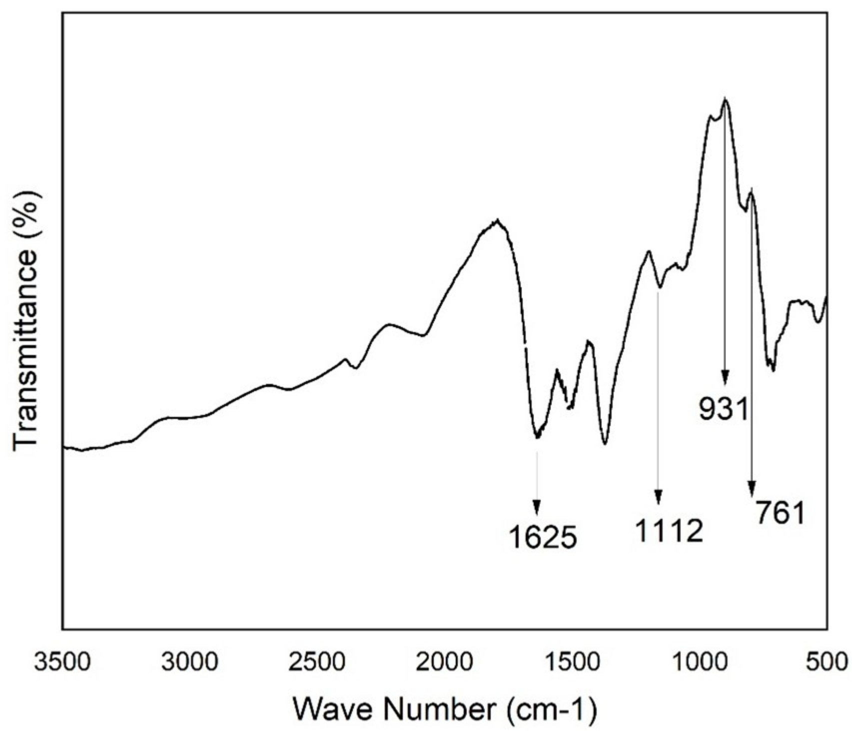


FIG. 3: FTIR spectrum of Ag_2O NPs.

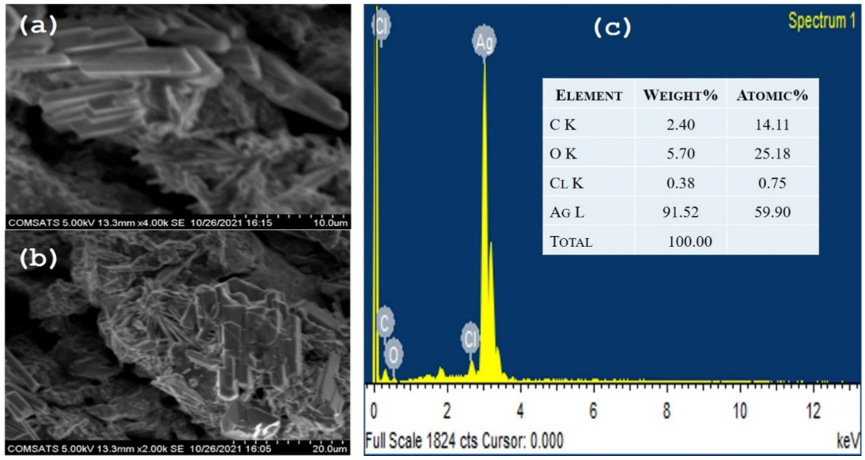


FIG. 4: SEM micrograph; (c) EDX of Ag_2O nanoparticles.

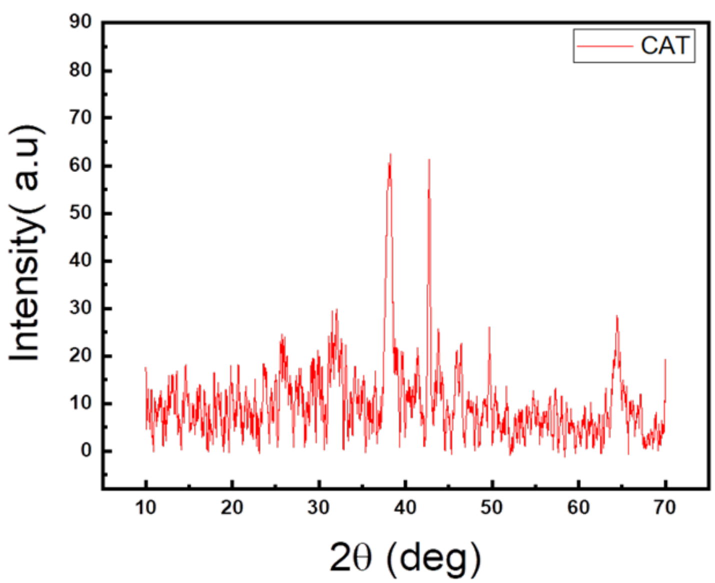


FIG. 5: XRD spectrum of Ag_2O nanocatalyst.

E. GENERAL PROCEDURE FOR SYNTHESIS OF 2-PHENYL BENZIMIDAZOLE USING AG2O NANOCATALYST

2-Phenylbenzimidazole was synthesized by reacting ortho-phenylenediamine (2.7 g) with benzaldehyde (1.5 mL) and benzoic acid (1.5 g) in the presence of Ag_2O nanocatalyst (0.15 g). The reaction was carried out in toluene (15 mL) as the solvent and was heated at 80°C under reflux for 3 hours. The resulting products formed precipitates, which were collected by filtration. The precipitates were then washed with solvent to remove impurities and dried in an oven for 3 to 4 hours. The yield and melting point of the product were recorded. A schematic representation of the synthesis is provided in Fig. 2.

2-Phenyl benzimidazole has also been synthesized using the procedure mentioned above but without the nanocatalyst. The yield and melting point of all prepared samples were recorded. A summary of the prepared benzimidazole (BZI) derivatives, along with their codes and reaction conditions, is presented in Table **??**. The characterization of the products was carried out using melting point measurements, Fourier transform infrared (FT-IR) spectroscopy, and 1 H NMR proton signals. The characteristic peaks observed are listed at the bottom of Table **??**. The Ag_2O nanoparticles (NPs) function as a heterogeneous catalyst by promoting the oxidation of aldehydes and enhancing the cyclization reaction through surface activation. The proposed mechanism involves the adsorption of reactants onto the NP surface, followed by oxidation-assisted imine formation and subsequent cyclization to produce benzimidazole derivatives. This catalytic efficiency is consistent with previous studies on metal oxide nanocatalysts[28,29]. Consequently, silver oxide nanoparticles act as Lewis acids, facilitating proton transfer and enhancing reaction kinetics, as illustrated in Fig. 2.

1. PHENYLBENZIMIDAZOLE USING BENZALDEHYDE IN TOULENE

$C_{13}H_{10}$ N_2; Brown Solid ($\sim 89\%$); mp 293-296 °C; FTIR; N-H 3200 $cm^{-1}C = C1645\ cm^{-1}$, $C = N1635\ cm^{-1}, -C - H925\&748\ cm^{-1}$. ^1H NMR $(CDCl_3): \delta 8.01-8.03(\ s, NH), 7.56-7.58(\ s,\ 3H), 7.42-7.43(\ s, 3H), 7.24-7.25(\ s, 3H), 6.71-6.74(\ d, 3H)$.

2. PHENYLBENZIMIDAZOLE USING BENZOIC ACID IN TOULENE

$C_{13}H_{10}$ N_2; Spice-Brown solid ($\sim 92\%$); mp 293-296 °C; FTIR; N-H 3200 $cm^{-1}C = C1649\ cm^{-1}, -C = N1631\ cm^{-1}, -C - H941\&744\ cm^{-1}$. ^1H NMR $(CDCl_3): \delta 8.07-8.09(\ d, NH), 7.55-7.59\ (t, 3H), 7.42-7.49(t, 3H), 7.24(\ s, 3H), 6.71-6.77(\ d, 3H)$.

III. RESULTS AND DISCUSSION

A. CHARACTERIZATION OF AG2O NANOCATALYST

The FTIR spectrum Ag_2O nanocatalyst prepared from the ginger extract is shown in Fig. 3. The characteristics peak of Ag-O appeared at 601 cm^{-1} and the rest of the peaks at 1112 cm^{-1}, 931 cm^{-1}, 859 cm^{-1}, 761 cm^{-1}, and 601 cm^{-1} are attributed to heterocyclic chemicals such as alkaloids, flavonoids, and alkaloids present in the extract. The stretching vibrations of $-C=C$ were assigned to the peak at 1652 cm^{-1} (aliphatic). The appearance of a peak at 1380 cm^{-1} confirms the presence of NO_3.

The silver oxide nanocatalyst was synthesized from ginger extract were scanned by scanning electron microscope. The micrographs (Fig. 4a and b) depicted remarkably homogenous spherical-tubular nanoparticles. Further, the nanoparticles show agglomeration due to strong surface interactions among them. The aggregated morphology of the particles can be seen in the micrograph shown in Fig. 4b. Similar morphology of the silver oxide nanoparticles synthesized by the green approach reported in the literature is in accordance with our results[28,30,31]. In Fig. 4c, the EDX analysis of Ag NPs is presented to demonstrate the elemental composition of the Ag(91.52%) and O(5.70%). The qualitative EDX patterns display the elemental composition, atomic percent, and weight percentage of the prepared nanocatalyst.

From the XRD spectrum of Ag_2O nanoparticles (Fig. 5) synthesized by green methodology, the characteristics peaks at 33°, 47°, and 58° correspond to plans 111,200 , and 220 , respectively confirming the face-centered cubic silver oxide nanoparticles. The 2 theta values of the XRD spectrum of silver oxide nanoparticles are quite close to the values reported in the literature[28,30].

B. ANTI-BACTERIAL SCREENING

By adopting the well diffusion method with six wells per plate, the antibacterial activity of benzimidazole derivatives was determined using four compounds. The bacterial culture was grown in mueller hinton agar media, using 5% DMSO solvent for three different concentrations of prepared derivatives at 37°C incubation for 48 hours. The four different concentrations of 20, 50, 80, and 8mg/mL of 2-phenyl benzimidazole prepared from two different routes were used to find the antibacterial activity against Escherichia colli. It can be clearly seen from Fig. 6, the compounds AT-2 and CT-2 prepared in the presence of Ag_2O nanocatalyst show the maximum zone of inhibition i.e, (25 ± 0.23 mm) and (16 ± 0.30 mm) at 50 and 80mgmL^{-1} concentration, respectively. Apart this, the compound prepared using nanocatalyst shown better antibacterial activities as well as the compound also without catalyst. The different values of zone of inhibition under different concentrations are listed in Table I.

IV. CONCLUSION

The research demonstrates that the use of Zingiber officinale root extract is an effective method for synthesizing Ag_2O nanoparticles, as evidenced by several analytical techniques such as FTIR, XRD, and SEM analyses. These techniques confirm the successful synthesis of Ag_2O nanoparticles and their morphology and crys-

TABLE I: Antibacterial activity of BZI derivative with & without nanocatalyst.

Bacterial spp.	Concentration ($mgmL^{-1}$)	Zone of inhibition (mm) AT-2	CT-2	AT-1	CT-1
E.Colli.	20	20 ± 0.38	9 ± 0.54	20 ± 0.38	-
	50	25 ± 0.23	12 ± 0.68	27 ± 0.29	3 ± 0.1
	80	-	16 ± 0.30	-	3 ± 0.1
	8	18 ± 0.56	-	20 ± 0.34	-

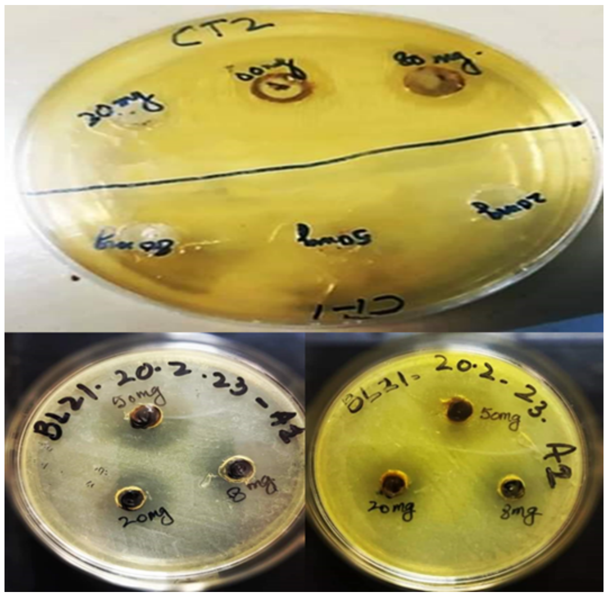


FIG. 6: Antibacterial screening of BZI derivatives.

tal structure. The study also reveals that the synthesized Ag_2O nanoparticles can be used as a catalyst for the synthesis of benzimidazole derivatives. The reaction involves the use of *o* phenylenediamine and aldehyde/benzoic acid in toluene solvent and occurs at room temperature. The reaction yields were found to be excellent, indicating the potential of Ag_2O nanoparticles as an efficient and cost-effective catalyst for organic synthesis. In a nutshell, the findings of this research hold significant promise for the development of new and sustainable catalytic systems using plant extracts as reducing agents for nanoparticle synthesis. Additionally, the use of Ag_2O nanoparticles as a catalyst for organic synthesis could provide an eco-friendly alternative to traditional chemical catalysts, which may have toxic byproducts or require harsh conditions.

DECLARATION OF COMPETING INTEREST

The authors have no conflicts to disclose.

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