

Research Article



Synthesis of CuO Nanoparticles by using Leaf Extracts of *Melia azedarach* and *Morus nigra* and their Antibacterial Activity

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Abstract | Nanoparticles are gaining popularity due to their increasing biomedical applications as anti-bacterial, anti-fungal and anti-cancer agents. In this work leaf extracts of *Melia azedarach* (commonly known as dharaik) a species of family Meliaceae and *Morus nigra* (commonly known as Shahtoot) family Moraceae were used for bio reduction of copper ions to CuO nanoparticles in place of harmful and expensive chemical/physical methods. A cheaper, simple to perform method giving high yield without by products is employed here. The leaf extract act both as reducing and capping agent. The atomic absorption shows a linear curve between concentration and absorbance. The uv-vis spectrum revealed the shift of absorption towards longer wavelength with the increasing concentration of plant extract. XRD indicates CuO Nps particle size is in between 14-20 nm. CuO nanoparticle show very significant antibacterial activity against four bacterial strains three gram negative (*Escherichia coli*, *Proteus mirabilis*, *Salmonella*) and one gram positive (*Clostridium tetani*). The minimum concentration of leaf extracts needed against these bacterial strains were also calculated. The present study indicated that *Melia azedarach* and *Morus nigra* leaf extracts can be used successfully for nanoparticles synthesis and CuO as significantly active antibacterial material. Antibacterial activity of these nanoparticles was observed by agar well diffusion method.

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Keywords | Nanoparticle, Leaf extract, XRD, Agar well diffusion method, *Escherichia coli*, *Clostridium tetani*, *Proteus mirabilis* and *Salmonella*

1. Introduction

Nanotechnology deals with the synthesis of particles and their structures ranging in size from 1-100 nm. Metal nanoparticle are quite significant due to their optical, electrical and electronic properties (Perez *et al.*, 2005; Sadri *et al.*, 2014; Maitinise *et al.*, 2017; Zhang *et al.*, 2017). They also have a prominent role as gas sensors (Korotcenkov, 2007; Zhou *et al.*, 2017), electrochromic devices (Argazzi *et al.*, 2004) and in solar cells (He *et al.*, 2012). For increasing their potential, multicomponent oxides are also

prepared (XU *et al.*, 2006; Zeng *et al.*, 2009; Wadkar *et al.*, 2013). Their biodegradable and biocompatible modifications resulted in reduction of their toxicity (Li *et al.*, 2007; Deng *et al.*, 2008).

Nanoparticles synthesis involve different methods (physical and chemical) e.g. synthesis of CuO nanoparticles by precipitation method, inverse micro emulsion system, Sol gel method etc. (Hashoosh *et al.*, 2014; Davarpanah *et al.*, 2015; Hasnidawani *et al.*, 2016). Unfortunately, these methods include the use of toxic chemicals and non-eco-friendly

by products (Ahmed *et al.*, 2015). Hence, it is the need of the day to opt an alternate environmental friendly synthetic route for obtaining nanoparticles using naturally occurring ingredients as reductants. Thus, Metal oxide nanoparticles by green method are gaining importance (Ahmed *et al.*, 2016). Nanoparticles are mainly classified as inorganic and organic. Inorganic Nps consists of either metal or metal oxides (e.g. NPS of Ag, Fe₃O₄, TiO₂, CuO and ZnO etc). Presently green chemistry involves their biosynthesis through microorganism and plant extracts (Sone *et al.*, 2015; Jayaprakash *et al.*, 2017). By using natural extract of *Aspalathus Linearis* crystalline perovskite ZnSnO₃ nanoclusters, NiO, Pd and PdO nanoparticles are biosynthesized (Mayedwa *et al.*, 2017; Mayedwa *et al.*, 2018). *Sageretia thea* and *Moringa Oleifera* natural extracts are used as chelating agent to prepare ZnO nanoparticle (Mayedwa *et al.*, 2017; Matinise *et al.*, 2017). Ag NPs were synthesized from aqueous *Eriobotrya japonica* leaf extract (Rao and Tang, 2017), fruit extract of *Capsicum frutescence* (Sweet pepper) (Shanker *et al.*, 2017) and *Mentha asiatica* mint extract (Sarkar and Paul, 2017). In addition to chemical synthesis (Heiligttag and Niederberger, 2013; Jabbar, 2016) Cu NPS can also be prepared by using natural ingredients (leaf extract) (Khan *et al.*, 2017). Organic NPS are mainly polymeric or lipids having 10nm to 1µm diameter (Couvreur, 1988). Liposomes, dendrimers, carbon nanomaterials and polymeric micelles are examples of organic nanoparticles.

Inorganic nanoparticles are preferred as antimicrobial agents (Loomba *et al.*, 2013; Jain *et al.*, 2014) as organic NPs cannot tolerate high temperature (e.g. quaternary ammonium compounds, N-halamine compounds and chitosan etc). The bactericidal effect of inorganic NPS has been attributed to their characteristic micron size less than the pore size of the bacteria and thus, they are capable of easily crossing the cell membranes without any hindrance (Deivasigamani *et al.*, 2015).

2. Materials and Methods

Due to the local availability and local medical use leaves of *Morus nigra* (shahtoot) and *Melia azedarach* (dharaik) were selected (herbarium of botany department LCWU). Powdered 10g/100ml of each aqueous leaf extract was boiled (80°C for 20 min), subjected to shaker (5c/s for 10 minutes), filtered and then refrigerated (4°C) for further experiments. 99.9%

pure CuSO₄·5H₂O solution (0.01M) of analytical grade was also used.

2.1 Synthesis of CuO Nanoparticles from plants leaf extract

For biosynthesis of nanoparticles, 10 mL of each of leaf extract was mixed with 100 mL of freshly prepared 1×10⁻² M aqueous copper sulphate pentahydrate solution in 250 mL Erlenmeyer flask under continuous magnetic stirring. Formation of CuO nanoparticles was accompanied with colour change (blue to yellow). These were then purified by repeated centrifugation method at 6,000rpm for 25 min. Later the CuO nanoparticles were dried in an oven at 80 °C for 5 hours.

Table 1 Showing the procedure for the preparation of different concentrations of *M. Nigra* and *M. azedarach*.

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Concentration	Procedure
5%	5mL of the Plant leaf extract was mixed with 95mL of the 0.01M CuSO ₄ Solution.
15%	15mL of the Plant leaf extract was mixed with 85mL of the 0.01M CuSO ₄ Solution.
25%	25mL of the Plant leaf extract was mixed with 75mL of the 0.01M CuSO ₄ Solution.

2.2 Procedure for antibacterial activity

For antibacterial activity agar well diffusion method was employed (Prabu *et al.*, 2015). Four bacterial strains were selected including *Escherichia coli*, *Proteus mirabilis*, *Salmonella* and *Clostridium tetani*. Different concentrations of both leaf extracts (5%, 15% and 25%) were checked and their zone of inhibition (ZOI) in cm was measured.

3. Results and Discussion

3.1 Atomic absorption spectrometry

The confirmation of CuO NPS was done by getting straight line between concentration and absorbance. Figure 1and2 shows the increase in absorption with increasing concentration of CuO nanoparticles with the gradual increase of leaf extracts concentration (5 to 25%) for *Morus nigra* (*M.nigra*) Figure 1(a, b) and Figure 2(a, b) for *Melia azedarach* (*M.azedarach*).

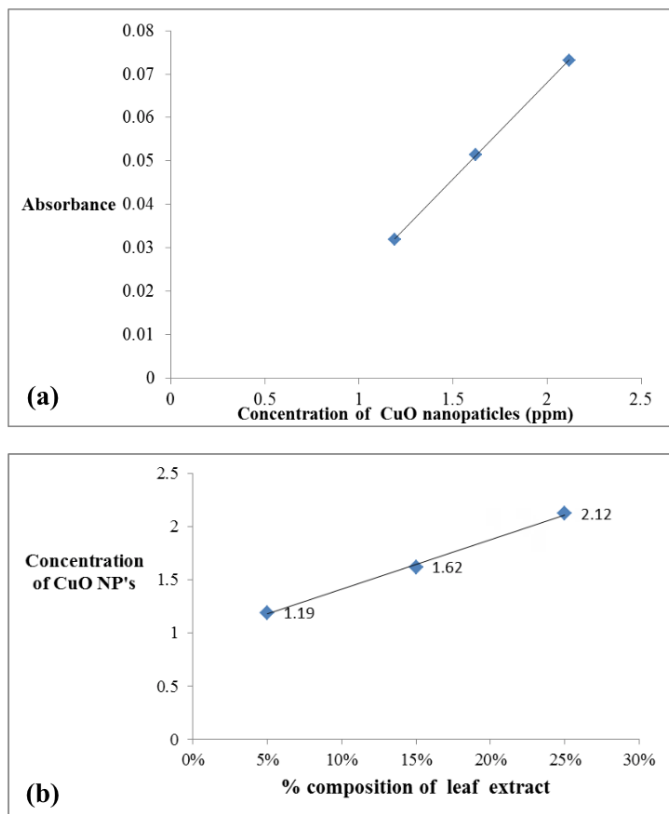


Figure 1: (a) Relation of CuO NPs concentration and Absorbance (*Morus Nigra*), (b): Effect on the synthesis of CuO NPs due to gradual increase in the *M. nigra* leaf extract concentration.

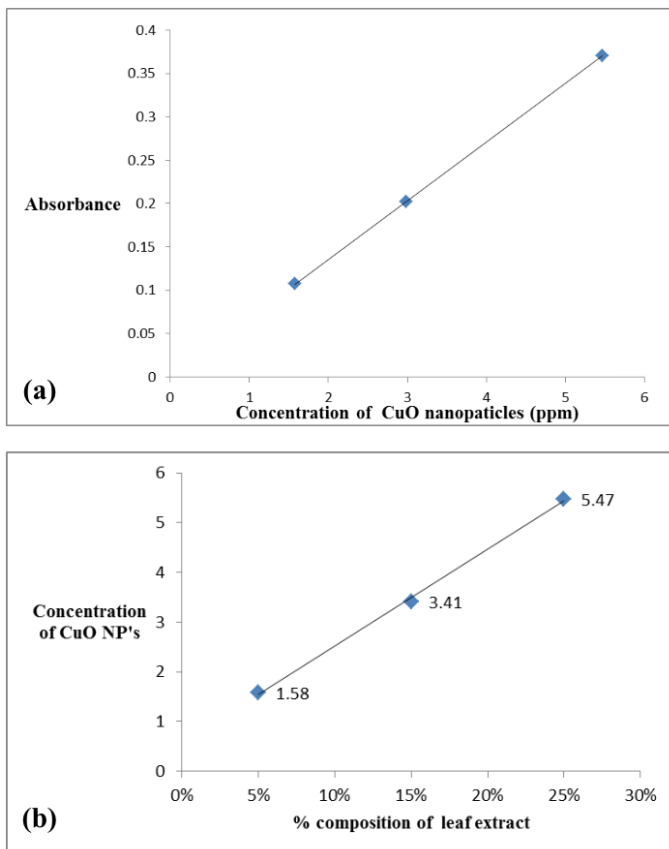


Figure 2: (a) Relation of CuO NPs concentration and Absorbance *Melia azedarach* (dharaik), (b): Effect on the synthesis of CuO NPs due to gradual increase in the *M. azedarach* leaf extract concentration.

fect on the synthesis of CuO NPs due to the gradual increase in concentration of *Melia azedarach* (dharaik) leaf extract.

3.2 Visual observation and UV-Vis spectroscopy

On addition of each leaf extract to aqueous CuSO_4 solution keeping its concentration same i.e. 0.01M, colour change was observed from pale blue to dark brown due to the conversion of Cu^{+2} ions to pure CuO nanoparticles Figure 3 (a, b) As the concentration of both leaf extracts (*morus nigra* and *Melia azedarach*) increases from (5-25%) the plasmon resonance bands shifts from 250-320nm (Capek, 2004) which is observed by UV-Vis spectroscopy Figure 4(a, b).

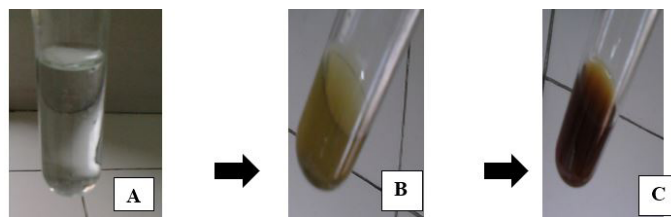


Figure 3 (a): A= pale blue (0.01M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$); B= greenish brown (aqueous leaf extract of *Morus nigra*); C= dark brown (addition of leaf extract into $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

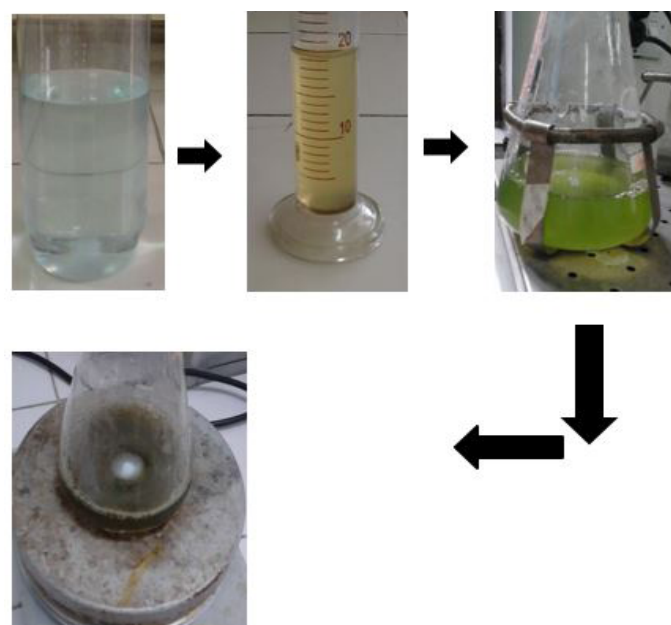


Figure 3 (b): Colour change from pale blue to yellowish green and finally dark brown (left to right) for *Melia azedarach*.

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

Three prominent bands of the CuO nanoparticles synthesized from *Morus nigra* aqueous leaf extract was at 3630 cm^{-1} for OH, 2158 and 2029 cm^{-1} for $\text{C}\equiv\text{C}$ and 1024 cm^{-1} is because of C-O stretching Figure 5(a).

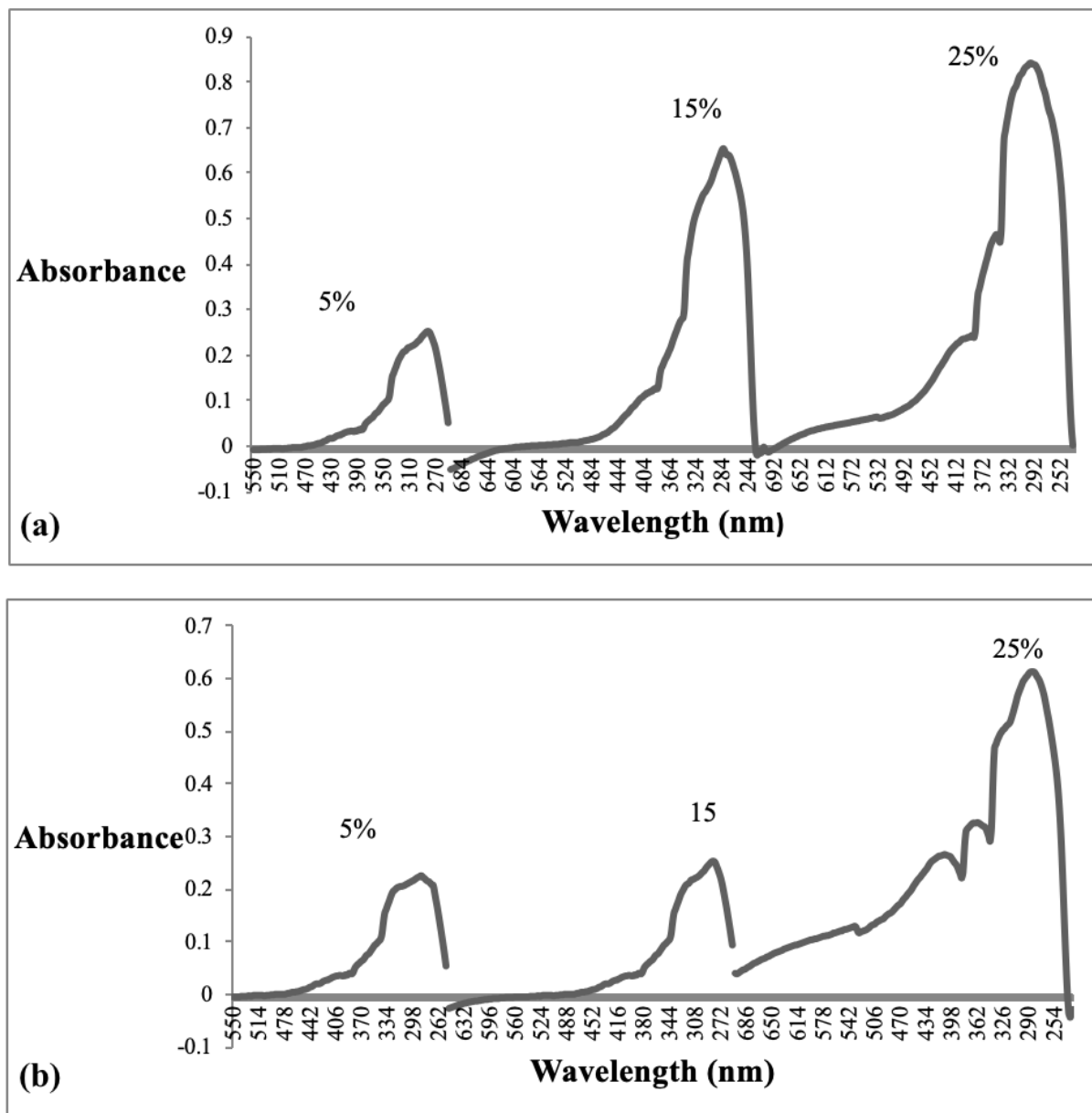


Figure 4: An increase in absorbance is observed with *Morus nigra* (a) and *Melia azedarach* (b) increased leaf extract concentration i.e. 5% to 25%.

For *Melia azedarach* Figure 5(b) a wide OH band is observed at 3352 cm^{-1} , C \equiv C peak at 2360 cm^{-1} , C=C group appeared as a weak band at about 1650 cm^{-1} . The peak for CuO bond stretching was observed at $507, 543\text{ cm}^{-1}$ (Matheswari et al., 2018). Carbonyl group and proteins from leaf extract can bind metal, forming a covering on metal nanoparticles. These results shows that some of the bioorganic compounds (leaf extract) played a double role i.e a strong covering and reducing agent (Prabu and Johnson, 2015).

3.4 X- Ray diffraction studies

The XRD results of CuO nanoparticles synthesized from *Morus nigra* exhibits peaks at $(32.75^\circ, 35.54^\circ, 38.57^\circ$ and 48.67° corresponding to planes $\{110\}$,

$\{002\}$, $\{200\}$, $\{202\}$) and for *Melia azedarach* exhibits peak at $(32.79^\circ, 35.49^\circ$, and 37.71° for $\{110\}$, $\{002\}$ and $\{200\}$) shows their monoclinic nature Figure 6(a, b).

The diffraction angle at 27.67° may be attributed to Cu nanoparticles instead of CuO nanoparticles. Thus, from these observations it may be concluded that some of the Cu NPs were so stable that they were not oxidized to form CuO nanoparticles.

All the peaks observed are due to Cu NPS and chances of independent crystallization of covering agent is no more because of using centrifugation process done for purification of NPS.

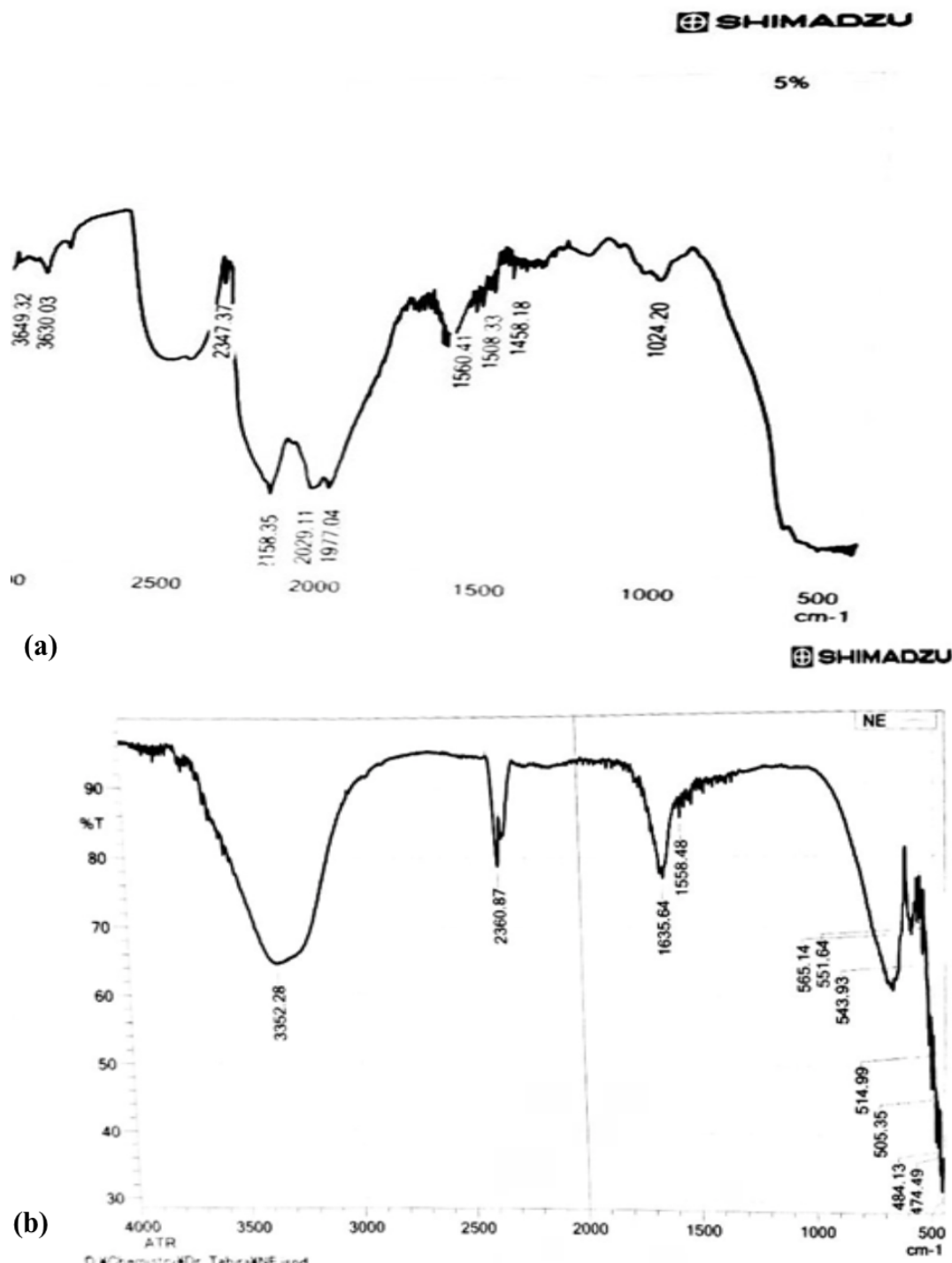


Figure 5: FTIR spectrum of CuO nanoparticles synthesized from *M. nigra* (a) and *Melia azedarach* (b) leaves extract.

The average nanoparticles size calculated (Debye–Scherrer equation) was 14–20 nm, which may indicate a high surface area-to-volume ratio of nanocrystals.

3.5 Antibacterial activity

Antibacterial activity of CuO nanoparticles was observed against four bacterial strains (gram positive and gram-negative bacteria) [Table 2](#).

Table 2: Showing different bacterial strains.

Gram Negative	Gram Positive
<i>Escherichia coli</i> ATCC 8739	<i>Clostridium tetani</i> ATCC 10779
<i>Proteus mirabilis</i> ATCC 25933	
<i>Salmonella</i> ATCC 700623	

3.6 Activity of CuO nanoparticles from *Morus nigra* and *Melia azedarach* leaf extract

25% (max conc.) showed maximum activity for both *M. nigra* and *M. azedarach* Figure 7(a, b, c, d, e, f). For *M. nigra* 1.2cm zone of inhibition (ZOI) for *Escherichia coli*, 0.6cm for *Clostridium tetani*, 1.3 cm for *Proteus mirabilis* and 1.2cm ZOI for *Salmonella* was observed Table 3. For *M. Azedarach* 1.6cm inhibition for *Escherichia coli*, 0.7cm for *Clostridium tetani*, 0.4cm for *Proteus mirabilis* and 1.1cm inhibition was against *Salmonella* Table 3.

Table 3: Evaluation of Antibacterial activity of CuO nanoparticles synthesized from *Morus nigra* and *Melia Azedarach*.

Sr. No	Bacterial Isolates	Zone of Inhibition in cm					
		M. Nigra			M. Azedarach		
		5%	15%	25%	5%	15%	25%
1	<i>Escherichia coli</i>	0.7	1.0	1.2	0.6	0.9	1.6
2	<i>Clostridium tetani</i>	0.3	0.4	0.6	0.3	0.5	0.7
3	<i>Proteus mirabilis</i>	0.5	0.9	1.3	0.2	0.3	0.4
4	<i>Salmonella</i>	0.6	0.9	1.2	0.3	0.7	1.1

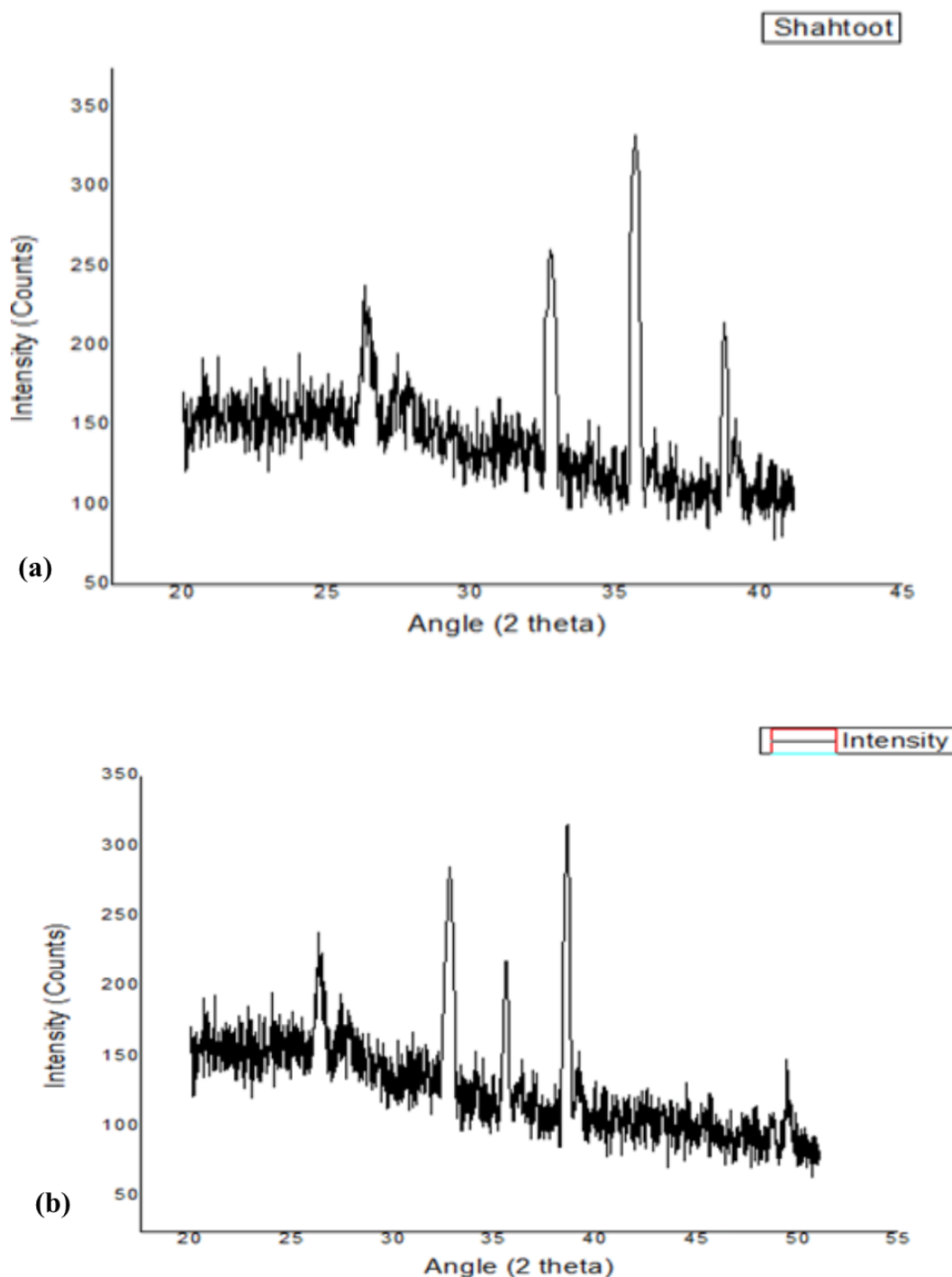


Figure 6: XRD plot of CuO NPs synthesized from *M. nigra* (a) and *Melia azedarach* (b) leaf extract.

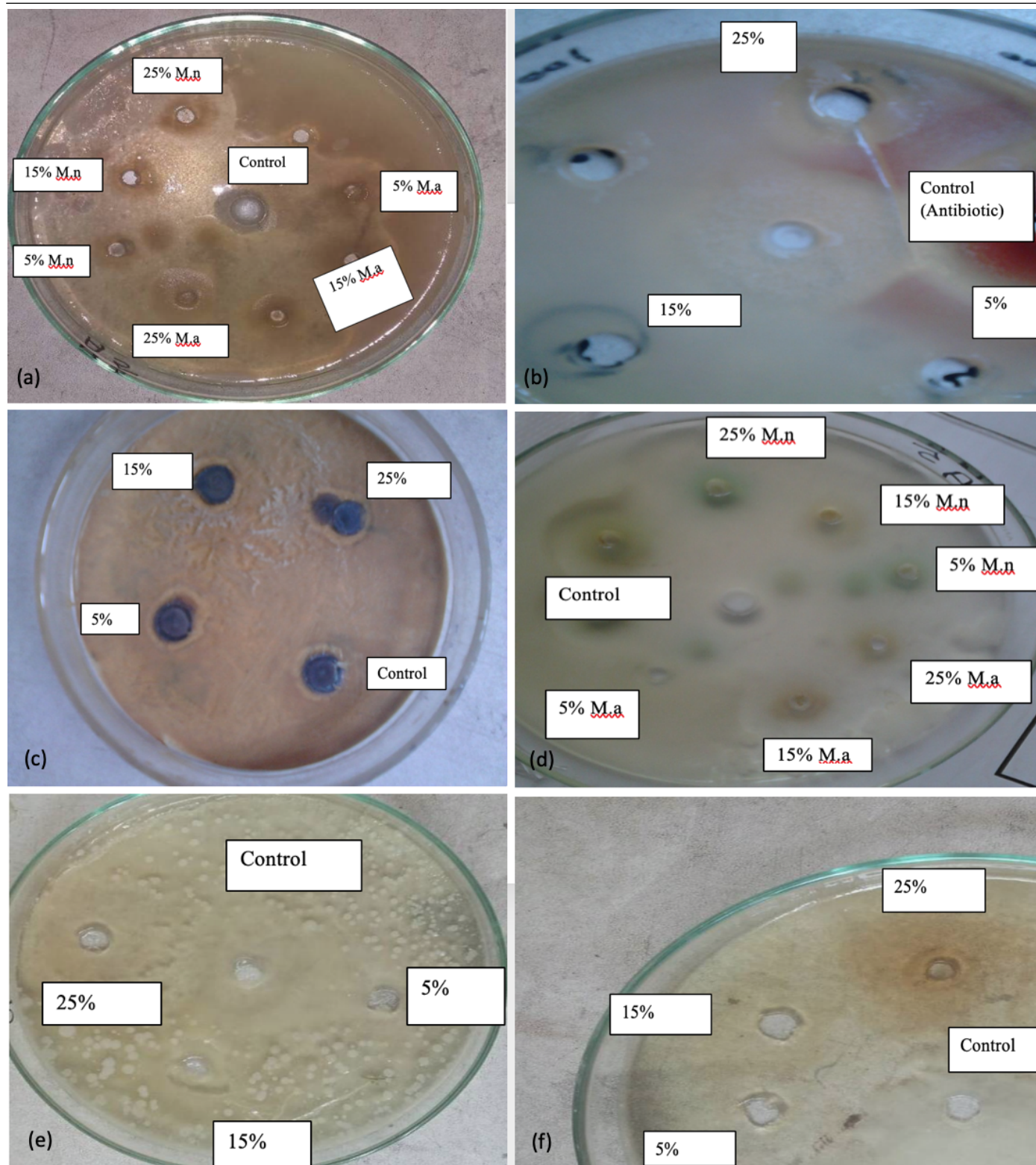


Figure 7: Showing the antibacterial results of CuO NPS synthesized from *Morus nigra* (M.n) and *Melia azedarach* (M.a) leaf extracts against a) *Escherichia coli* b) *Clostridium tetani* (M.n) c) *Clostridium tetani* (M.a) d) *P. mirabilis* e) *Salmonella* (M.n) f) *Salmonella* (M.a).

Conclusion

This study concludes that CuO NPS can be prepared from eco-friendly synthesis by using leaf extracts as natural ingredients. Confirmation of CuO nanoparticles was done with FTIR, colour change,

Atomic Absorption Spectroscopy, UV/Vis spectra and X-ray Diffraction studies. Thus, the leaf extracts of *Morus Nigra* (Shahtoot) and *Melia Azedarach* (dharaiik) successfully worked as natural ingredient. Antibacterial studies proved CuO NPs as good inhibitor against three gram negative and one gram positive strain by

measuring their Zone of inhibition. The Minimum inhibitory concentration of both the extracts were 5%. Thus, the above method is natural, significant, simple, low cost and producing no by-products.

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Author's Contribution

Tahira Moeen Khan: Concept and design of study.

Tahira Moeen Khan, Amat ul Mateen: Data acquisition, analysis and interpretation.

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