

RESEARCH PAPER

## Ziziphus mauritiana mediated synthesis of copper and nickel nanoparticles for comparative efficacy in biological water purification

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### ABSTRACT

The burden of life on the earth is the source of biological contamination in water. Nanotechnology has promising contributions in control of microbial contaminations, and medicinal plants further increase these properties. In this study, copper acetate and nickel oxide nanoparticles were synthesized using 1 mM solution of each with *Ziziphus mauritiana* leaves extract as the reducing agent. Nanoparticles were characterized through UV-Vis spectroscopy and scanning electron microscope, and antimicrobial properties were determined through disc diffusion method. Copper and nickel nanoparticles were adsorbed on filter paper strips and used in biological water purification. The viable bacterial count of water pre and post treatments were analyzed statistically. Absorbance peaks were recorded at 650 nm for copper acetate nanoparticles, and at 250 nm for nickel oxide nanoparticles. The size of nanoparticles, calculated using SEM, was up-to 47.90 nm for copper acetate and 48.40 nm for nickel oxide at resolution of 15kv x 60,000. The application of nanoparticle-coated strips in water indicated a significant reduction of 24% and 18% in total plate count of water samples during the first 2 h of treatment. Study concluded the antimicrobial properties of copper acetate and nickel oxide nanoparticles and paved a path for use of nanoparticles-coated membranes in filtration units for biological purification of water.

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### INTRODUCTION

About two-third of total earth's surface is covered by water and approximately 75 percent of human body consists of water. This clearly signifies that water is one of the most important and prime resources of life on earth planet. Estimated 70% of worldwide water is utilized in irrigation, 22% is being used by industry, and 8% is used for

domestic purposes. Water is the largest natural resource on earth, but about only 1% of this water is available for human utilization as fresh water [1]. With development of industries and growing urbanization, natural resources are under danger of being depleted. This global issue affects each continent, and as far as potential impact is concerned, it has been listed as one of largest global risks over next decade by the World Economic Forum in 2019.

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About one-third of worldwide population is facing severe water scarcity condition at least one month of year [2]. Adequate supply of clean and fresh water for human consumption has become a major concern due to contamination of water resources by organic and inorganic pollutants, heavy metals and pathogens leading to water pollution and water-borne diseases [3]. The microbial contaminations in ambient water bodies are a major concern at the global scale. Presence of waterborne pathogens in all water resources is a limiting factor in the provision of safe drinking water. The improvement of water resources is one of eight Millennium Development Goals (MDGs) of the United Nations. World water assessment program is working to supply clean water for 1.5 billion people [4]. Increasing population and improper sewage systems in developing countries are sources of biological water pollution. The number of bacterial and viral pollutants in water resources are increasing leading to infectious disease. Previously, many different conventional methods for purification of water were being used all over the world. Some of those important methods are filtration, gravity separation, chlorination, ozonation and ultraviolet treatments. Infectious pathogens can be removed by filtration by transforming their phase, however, this results in production of highly dense sludge that is poisonous and not disposable. Chlorination also proves as less effective due to production of some highly resistant pathogens and carcinogenic by-products [5]. By-products formed by ozonation are comparatively less in number. Ozonation is costly and when bromide ion in water reacts with ozone, harmful bromate is produced. Similarly, ultra-violet treatment and other conventional methods are less effective and form toxic by-products and these methods are costly too [6]. The development in nanotechnology has proved its capability for providing innovative methods for wastewater treatment [7]. Nano-adsorbents are formed using elements possessing high adsorption capacity and the nanoparticles used for them must be non-toxic, have high capacity for adsorption and also have the ability to remove heavy metals at low concentration [3]. Antibacterial properties of metal nanoparticles make them suitable candidates in the purification of water from bacterial contaminants [8]. The properties of metal nanoparticles are further enhanced with medicinal plant extracts as reducing agents. Keeping in view the properties of nanoparticles and the need of purified drinking water for human consumption, the present study is

designed for biological purification of water using nanotechnology.

## MATERIAL AND METHODS

### *Green route to synthesize copper and nickel nanoparticles*

The leaves of *Ziziphus mauritiana* were identified and collected from the botanical garden of the University of Agriculture, Faisalabad. A 10 g coarse powder of *Ziziphus mauritiana* leaves was added in 100 mL of the double distilled water and mixture was boiled for 30 minutes at 80°C. The extract was filtered and added in 1:9 ratio of 1 mM aqueous solution of  $\text{Cu}(\text{CH}_3\text{COO})_2$  and NiO separately. The colloidal mixture was heated at 60°C for 40 minutes with continuous stirring in the shaking water bath. A 20 mL of colloidal mixture was separated for further studies while the rest was dried in the oven. The fine black colored powder was collected carefully and stored in an amber colored bottle for further characterization [9].

### *Characterization of Copper and Nickel Nanoparticles*

The prepared copper acetate and nickel oxide nanoparticles were characterized using Ultraviolet-visible spectroscopy at 200 to 800 nm. The size of copper acetate and nickel oxide nanoparticles, and morphology were studied through scanning electron microscope. The nanoparticles in sterile distilled water suspension were processed for SEM analysis, a small drop was fabricated onto the electric stubs and water was evaporated completely before imaging [10].

### *Formation of nanoparticle composite with cellulose filter paper*

The cellulose filter paper (Whatman No. 1) was punched to prepare of 1X4". Filter paper disc and strips were pre-treated with 0.3M NaCl solution for five minutes in the petri dish. After 5 minutes in NaCl solution, both disc and strips were dried and placed separately in 20 mL of copper acetate and nickel oxide colloid for 24 h. The discs and strips were washed with distilled water and dried to remove loosely bound nanoparticle [11].

### *Antimicrobial efficacy of nanoparticles*

The filter paper discs containing 5µg of copper acetate and nickel oxide nanoparticles were tested against *Staph. aureus* and *E. coli* cultures procured from Institute of Microbiology, UAF. Commercially available antibiotic discs were used as standard in

the test plates. The culture plates were incubated for 18 h and results were observed [12].

*Application of nanoparticle-coated antimicrobial strips in water treatment*

A 50 mL of ground and tap water was collected in sterile beakers and flasks. Total viable count of water samples was done using the pour plate method before treatment with nanoparticle-coated filter paper strips. The filter paper strips coated with copper acetate and nickel oxide nanoparticles were placed in the test tubes for 2-6 h at room temperature. After every 2 h, the samples were processed for pour plate method and results were recorded [13].

**RESULTS**

The successful synthesis of copper acetate and nickel oxide nanoparticles was confirmed through UV visible spectrophotometer and scanning electron microscope. The absorbance peaks of copper acetate and nickel oxide nanoparticles were detected at different wavelengths. A significant absorption peak ranging from 550 nm to 650 nm was detected for copper acetate nanoparticles and a peak absorption was observed at 650 nm as

shown in Fig. 1, whereas, an absorption peak of 250 nm to 350 nm was significant for nickel oxide nanoparticles and a peak was observed at 250 nm as depicted in Fig. 2.

*Scanning electron microscopy of copper and nickel nanoparticles*

Scanning electron microscope was used for the size and morphological characterization of copper acetate and nickel oxide nanoparticles. Digimizer software was used to analyze the particle size of copper acetate nanoparticles at different resolutions of SEM. Fig. 3 is indicating copper acetate nanoparticles 0.2 μm at resolution of 15kv x 60,000. The micrographs attained from Digimizer are presenting minimum and maximum size of nanoparticles evaluated. In the photomicrographs of Digimizer, mean particle size and area along with its intensity and length are indicated. At the resolution of (15kv x 60,000), average size of copper acetate nanoparticles was calculated to be 33.8 nm, while the minimum and maximum sizes of nanoparticles were 22.3 nm and 47.9 nm, respectively, as indicated in the micrograph Figs. 4. and 5 depicts a photomicrograph of 0.2 μm size distribution of nickel oxide nanoparticles at 15kv x

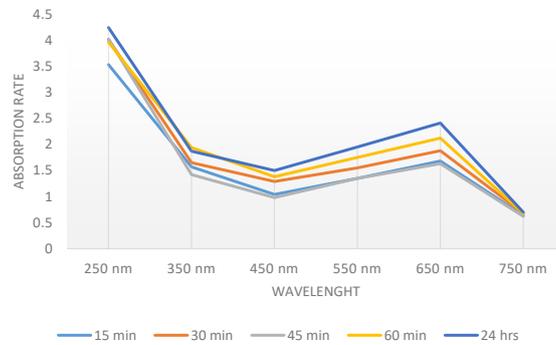


Fig. 1. Peak absorbance of copper acetate Nano particles at 650nm

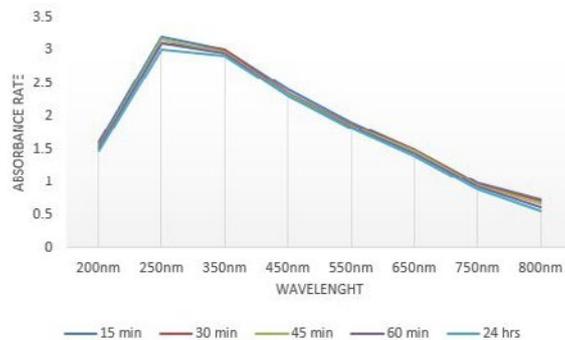


Fig. 2. Peak absorbance of Nickel oxide Nano particles at 250 nm



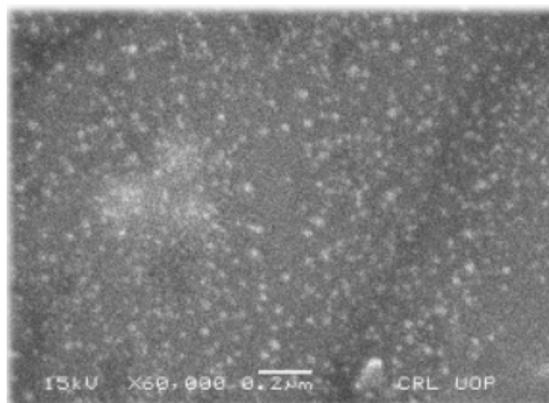


Fig. 3. SEM photomicrograph of copper nanoparticles at 15 kv x 60,000

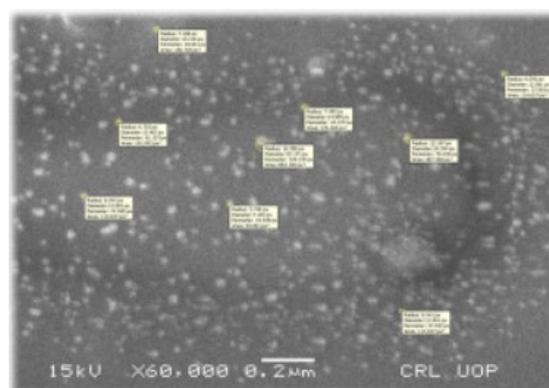


Fig. 4. Photomicrograph of Digimizer, mean particle size and area along with its intensity and length of copper acetate nanoparticles at resolution of 15 kv x 60,000

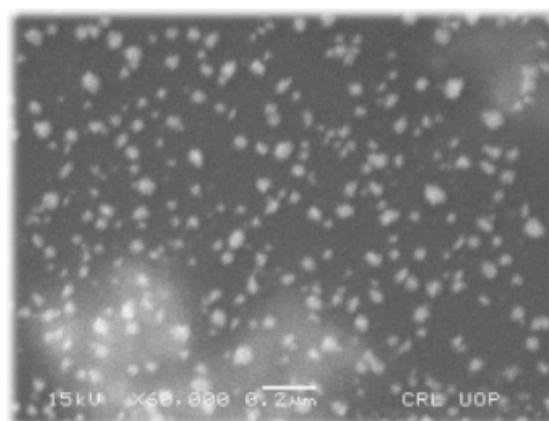


Fig. 5. SEM photomicrograph of nickel nanoparticles at 15 kv x 60,000

60,000. At resolution of (15kv x 60,000), the average size of nickel oxide nanoparticles was calculated to be 17.9 nm, while the minimum and maximum sizes of nanoparticles were 16.1 nm and 48.4 nm, respectively, as indicated in the micrograph

obtained from Digimizer described in Fig. 6.

#### *Antimicrobial efficacy of nanoparticles*

Two bacterial cultures (*Staph. aureus* and *E. coli*) on the MH agar were tested for antimicrobial activity

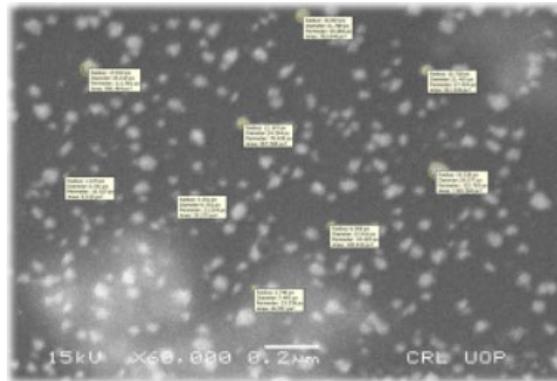


Fig. 6. Photomicrographs of Digimizer, mean particle size and area along with its intensity and length of nickel oxide nanoparticles at resolution of 15 kv x 60,000



Fig. 7. Lawn culture of *Staph. Aureus* indicating zone of inhibition around  $\text{Cu}(\text{CH}_3\text{COO})_2$  nanoparticle coated discs and standard antibiotic discs after 18 hrs of incubation at 37 °C



Fig. 9. Lawn culture of *Staph. Aureus* indicating zone of inhibition around NiO nanoparticle coated discs and standard antibiotic discs after 18 hrs of incubation at 37 °C



Fig. 8. Lawn culture of *E. coli* indicating zone of inhibition around  $\text{Cu}(\text{CH}_3\text{COO})_2$  nanoparticle coated discs and standard antibiotic discs after 18 hrs of incubation at 37 °C



Fig. 10. Lawn culture of *E. coli* indicating zone of inhibition around NiO nanoparticle coated discs and standard antibiotic discs after 18 hrs of incubation at 37 °C

of copper acetate and nickel oxide nanoparticle coated discs. The nanoparticle coated discs along with control positive antibiotic discs were observed

after 18 h of the incubation. The Figs. 7 and 8 indicate the results of copper acetate nanoparticles against *Staph. aureus* and *E. coli*, while Figs. 9 and

10 indicate zone of inhibition developed by nickel oxide nanoparticles on the lawn of *Staph. aureus* and *E. coli*. The plates indicate that *Staph. aureus* is more susceptible to both copper acetate and nickel oxide nanoparticles with a diameter ranging from 16-20 mm. *E. coli* was less susceptible to both copper acetate and nickel oxide nanoparticles coated discs with a diameter ranging from 8-11 mm. Copper acetate nanoparticles developed a zone of 11 mm on *E. coli* and 17 mm on the *Staph. aureus* culture plate, while nickel oxide nanoparticle coated disc developed a zone of 12 mm and 19 mm on the *E. coli* and *Staph. aureus* culture plates, respectively. The zone of no growth around standard antibiotic disc was also different for both culture isolates. *E. coli* was completely resistant to penicillin while *Staph. aureus* gave a zone of 12 mm against

penicillin. Zone of inhibition around gentamycin was 18 mm and 22 mm for *E. coli* and *Staph. Aureus*, respectively. Both of the culture *E. coli* and *Staph. aureus* isolates were susceptible to Cefoxitin with a diameter of 18 mm and 24 mm, respectively. Fig. 11 indicates the susceptibility percentages of *E. coli* and *Staph. aureus* for nanoparticle-coated discs in comparison with standard antibiotics.

*Purification of water through antimicrobial filter strips*

Antimicrobial filter strips coated with copper acetate and nickel oxide nanoparticles were placed in tap water samples and results were recorded after every 2 h till 6 h. Fig. 12 describes status of viable bacterial count in the water samples treated with nanoparticle-coated strips. At 0 h, a high

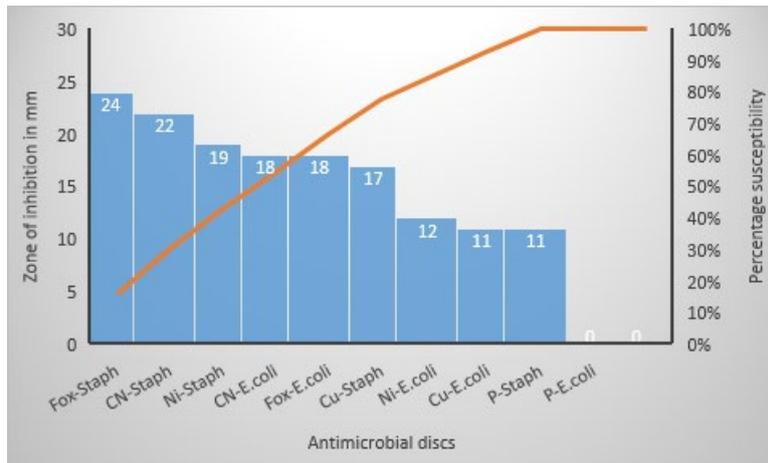


Fig. 11. Graphical presentation of nanoparticle coated antimicrobial discs and standard antibiotic discs against *Staph. aureus* and *E. coli*

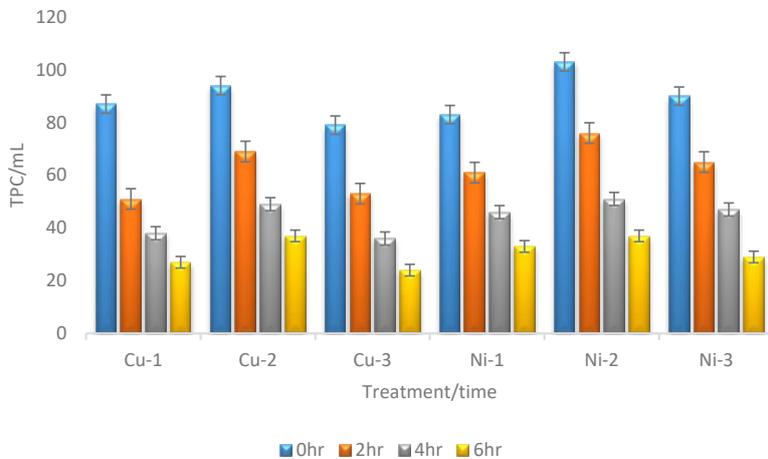


Fig. 12. Graphical presentation of total plate count per ml in the water samples treated with copper and nickel nanoparticles for 6hrs



number of viable bacterial was recorded in 79 to 103 bacterial/mL in six water samples. After first 2 hours of water treatment, a significant decrease in bacterial count was observed. A 24% reduction in total viable count was recorded in the water samples treated with copper acetate nanoparticle-coated strips, while after 4 h and 6 h of treatment, 4% and 1.7% more reductions were observed that were also significant.

In case of water samples treated with nickel oxide nanoparticle-coated strips, the reduction in total viable count was significant (18%) after first 2 h of incubation, while after 4 h and 6 h further 3.8% and 2.8% reductions in total viable count were recorded. The reduction in viable bacterial count during 4-6 h was non-significant. The results are in favor of copper acetate nanoparticles for efficient purification of water samples by reducing more viable count in short time compared to nickel oxide nanoparticles.

## DISCUSSION

Water is an important element for existence of life on earth. World health organization (WHO) recommended clean water free of dirt, turbidity and colorless, odorless for drinking [2]. Water reserves are being depleted and contaminated with increase in worlds population, especially developing countries are suffering from acute shortage of clean drinking water, whereas 95% population of developed countries is enjoying safe and healthy drinking water. The conditions in Pakistan are equally worse where only 25% of individuals have access to safe water and this 25% is divided into 70% rural and 30% urban people [14]. Water pollution is associated with mixing of human and animal waste in the water wells along with increased industrial and agriculture wastes. Biological contamination of drinking water is directly linked with gastrointestinal and hepatic diseases [15]. Tiwari et al., 2007, published a review article indicating vast applications of nanoparticles in waste water treatment [16]. The copper and nickel metals were prime focus for synthesis of nano-scale particles in future for purification of waste water through nanoparticles. The important point of discussion in that study was the combined treatment of ultrasonic irradiations with nanoparticles to fight against coliform bacteria.

Biological contamination increased coliform count in water where fecal *E. coli* is prominent bacteria. Since past, a number of technologies have been adopted for cleaning the drinking water including chlorination. All of these technologies

have their pros and cons. Present study was designed to clean the drinking water through nanoparticle-coated antimicrobial filter paper strips.

Nanoparticles were synthesized from two metal compounds (copper acetate and nickel oxide) with *Ziziphus mauritiana* leaves extract as a reducing agent. A dark colored colloidal mixture of copper acetate and nickel oxide with *Ziziphus mauritiana* leaves extract was obtained after heating at 60 °C for 40 min. The results of change in color of reaction mixture were in accordance with the study performed by Anjum et al. [17]. The potential of *Ziziphus mauritiana* leaves extract to reduce the metal ion suspension for synthesis of nanoparticles was efficiently utilized. The synthesis of nanoparticles through green route was also compared with the study performed by Kaur et al. [18], that proved reduction and change of color as primary parameter for copper acetate nanoparticle synthesis. The prepared nanoparticles of copper acetate and nickel oxide were characterized through UV-visible spectrophotometer. Absorption peaks of copper acetate nanoparticles were recorded at 650 nm which were in accordance with the study performed by Ashtaputrey et al. [19], indicating absorption peaks of copper acetate nanoparticles at 340 nm justifying an increased absorption peaks as point of enhanced synthesis of nanoparticles. Sharma et al. [20] characterized copper nanoparticles at 248 nm while Fernando and Gurulakshmi, [21] characterized copper nanoparticles through UV-Vis spectrophotometry at 301 nm. The UV-Vis spectrophotometer characterized the absorption peaks of nickel oxide nanoparticles at 250 nm to 300 nm, with the maximum peak at 250 nm, similar to the results of study performed by Sudhasree et al. [22], presenting absorption peaks of 250 nm. Nanoparticles of copper acetate and nickel oxide were also characterized using scanning electron microscopy at three different resolutions of (15kv x 1,00,000), (15kv x 60,000) and (15kv x 30,000) for 0.1µm, 0.2µm and 0.5µm size distribution of particles. The particles size of copper acetate nanoparticles was calculated up to 47.90 nm at resolution of (15kv x 60,000). The results of SEM for copper acetate nanoparticles were correlated with characterization performed by Galan et al., [23], describing nanoparticles of 6-61 nm. Meanwhile, the SEM results for nickel oxide nanoparticles in current study indicated a particle size of 48.40 nm at (15kv x 60,000) resolution. Suresh et al., [24], developed copper acetate nanoparticles of 5-40 nm with an

average of 20 nm that are near to the nanoparticles developed in current study. Similar results of SEM for nickel oxide nanoparticles were presented by Mariam et al. [25], showing nanoparticles from 22 nm to 44 nm through different characterization methods including SEM. The nanoparticles were further evaluated for their antimicrobial efficacy. Previous research reported copper acetate nanoparticles more effective against Gram negative bacteria, but in present study copper acetate as well as nickel oxide nanoparticle-coated strips were applied for biological water purification. Mahesh and Satish, [26] reported antibacterial properties of *Z. mauritiana* leaf extract at 14 mm and 18 mm against *E. coli* and *Staph. aureus* at concentration of 100 µg per 5 mm disc. The antimicrobial properties of nickel oxide nanoparticles were correlated with the study performed by Khashan et al. [27] reporting enhanced antibacterial activity of NiO nanoparticles at 1000 µg/mL. The antibacterial efficacy of nanoparticles determined in current experimental conditions was under lower concentrations, but positive attitude of nanoparticles verified their ability to enhance the penetration power of antibiotics if used in combination with nanoparticles.

Gehrke et al., [28] focused on nano adsorbents for purifications of the waste water and highlighted the importance of nano-engineered materials for their application in water treatments. Currently the same concept was used for the synthesis of nanoparticle adsorbed cellulose strips and their applications in water purifications. Nanoparticle-coated filter paper strips were placed in tap water at room temperature and results of total viable bacterial count were recorded through total plate count assay. A significant reduction in total plate count was recorded from 2-6 h in the water samples immersed with copper acetate and nickel oxide nanoparticle-coated filter paper discs. Initially a significant reduction in total viable count was recorded up to 24% and 18% in the water samples treated with copper acetate and nickel oxide nanoparticles coated strips. While during 4-6 h of treatment the reduction in viable count of sample was significant for copper acetate and non-significant for nickel oxide nanoparticles. The time laps indicated decrease in efficiency of nanoparticles to reduce the viable bacterial count probably due to continuous exposure of nanoparticle-coated strips with the light. Mustafa, [29] applied nanoparticles for purification of water. In that study, silver nanoparticles were synthesized using fungal species and characterized through TEM, UV-Vis

spectroscopy and FTIR. The results of present study are in correlation with previous studies for excellent antibacterial properties against both Gram positive and negative bacteria.

Current research evidenced antimicrobial efficacy of copper acetate and nickel oxide nanoparticles in biological purification of water. Lu et al., [30] also presented an articles on nano sized particles and their successful use in waste water treatments. Nano composites were discussed in details for their potential as antibacterial and current study showed that nano based composites/ adsorbents have potential to clear high coliform count from the water.

## CONCLUSION

Many studies have been conducted to technologically improve the techniques of water treatment. Previous studies highlighted the importance of nano based materials for their possible use against various pathogens. The ability of nanoparticles in purification of water has been studied after adsorption of nanoparticles on cellulose paper strips. The promising results were achieved in clearing water from pathogenic coliform. Copper acetate and nickel oxide nanoparticles provided an effective way of water treatment in the form of adsorbed filter paper strips. The reduced coliform count of waterpost treatment of nanoparticle coated filter strips is indicative of antibacterial properties of nanoparticles leading to the foundation of a new idea to adsorb these green synthesized nanoparticles on the cellulose paper. The nanoparticle-adsorbed cellulose paper can be further employed for purification of water at large scale. The present study is also providing an advancement of nanotechnology to use house filter after adsorption of nano sized particles that may reduce the coliform count from drinking water and help in combating deadly problems of gastrointestinal tract resulting from high coliform count.

## CONFLICT OF INTEREST

The authors declared to no conflict of interest.

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