ULTRA-SMALL COPPER OXIDE NANOPARTICLES (UCUONPS): SWERTIA CHIRAYITA MEDIATED FACILE GREEN SYNTHESIS, PHYSICOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL EFFICACY

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ABSTRACT

The present work aims at the synthesis of ultra-small copper oxide nanoparticles (UCuONPs) in the diameter range 2-10 nm by using aqueous and alcoholic extracts of *Swertia chirayita* as a reductant and stabilizer with their possible use as an antibacterial agent against Gram-positive and Gram-negative bacterial strains.

The UCuONPs were characterized employing Fourier transform infrared spectroscopy, X-ray diffraction, UV-Visible spectroscopy, high-resolution transmission electron microscopy, scanning electron microscopy and energy-dispersive x-ray spectroscopy. The results confirm that the UCuONPs were successfully prepared by the interaction of copper (I) ions and *Swertia chirayita* plant extracts.

The as-prepared UCuONPs were further studied for their possible antibacterial activity against Gram-positive *(Staphylococcus aureus)* as well as Gram-negative (*Escherichia coli* and *Salmonella enterica*) bacterial strains. The efficacy of *Swertia chirayita* extract based UCuONPs were found to be more effective against Gram-positive than to Gram-negative bacterial strains suggesting their use in cases of resistant bacterial infections.

Keywords: antibacterial efficacy, *Swertia chirayita* extract, Ultra-small copper oxide nanoparticles (UCuONPs).

1 INTRODUCTION

Infectious diseases are one of the serious problems faced by public worldwide, mainly due to the emergence of antibiotic-resistant strains of Gram-positive and Gram-negative bacteria. Over the years, antibiotics had been used to fight these superbugs. However, antimicrobial resistant bacterial strains are the major problem faced by developing nations due to limited access to medical care and effective treatments. So, the need of the hour is to invest time and money in developing cost effective and prompt antibiotics to fight these resistant bacteria.

Nanotechnology is a boon for the scientific development in the 21st century particularly due to the synthesis of nanoparticles of specific size and shape. To date, worldwide researchers are now focussing on the development of metal nanoparticles due to their outstanding properties making them a potential candidate for multidisciplinary applications [1]. Metal nanoparticles owing to their specific characteristics have successfully been studied in various fields such as catalysis, optics, electronics, magnetics and antimicrobials [2, 3, 4]. The growing environmental concerns have provoked researchers to focus on the green

synthesis of useful chemicals in general and of metal nanoparticles in particular.

Biosynthesis of noble inorganic nanoparticles using microorganism is a busy area of research worldwide. Our blue planet is a copious source of predominant phototrophs as plants estimating approximately 2.5 to 5 lacs species on its surface [5]. Green synthesis of metal oxide nanoparticles using plant extracts and exploration of their antibacterial efficacy have been the subject of extensive research worldwide [6]. It is a one-pot, cheap and environmental friendly approach using phytochemicals of plants as reducing agent for reducing metal ions into metal nanoparticles. Among the various nano-metals explored so far, nanoparticles of silver, gold, copper, zinc, palladium, titanium, nickel, indium etc. have been prepared by using a wide variety of plant extracts.

Copper nanoparticles are of great interest because of their wide applications in heat transfer, sensors, antimicrobial agents etc. [7-16]. Ultra-small nanoparticles of size ranging from 1-10 nm are highly sensitive to both the composition and size of the particles and thus their controlled synthesis is an important area of research. There is not yet a well-established definition of ultra-small nanoparticles as defined in the various literature. Nanoparticles with the diameter less than 2 nm, 5-6 nm and isotropic nanoparticles with diameter 10 nm have been categorized as being ultra-small nanoparticles [17, 18, 19]. Although the size of the copper nanoparticles synthesized so far are generally in the range of 20-100 nm. Copper nanoparticles synthesized chemically have capping agent on the surface due to addition of the extra surfactant *in vivo* whereas green synthesis does not require any surfactant as the plant extract acts as reducing agent as well as capping agent [20]. Though lots of efforts have been invested in the synthesis of metal nanoparticles using the wide variety of plant extracts, however, a limited number of works have been reported for the plant mediated synthesis of ultra-small copper nanoparticles [21, 22].

Swertia chirayita is a valuable bitter tonic which is used as anti-diarrhoeal, anti-helminthic, anti-inflammatory, anti-leucorrhic, antipyretic, anti-rheumatic etc. It is also used to treat different types of fevers especially chronic and intermittent fevers, scabies and other skin diseases in the traditional medicine system. Besides, it acts as a remedy for bronchial asthma and in liver disorders [23-28]. It shows a significant antibacterial activity against resistant strain MRSA (Methicillin Resistant *Staphylococcus aureus*) and various Gram negative strains [29, 30].

Ultra-small copper oxide nanoparticles (UCuONPs) were successfully synthesized using surfactant templates and carbon nanotube scaffolding with citrate reduction [31]. Ultra-small copper nanoparticles having the diameter of <2 nm have also been synthesized using $CuCl_2.2H_2O$ as precursor by hydrazine reduction in the presence of citric acid and cetyltrimethylammonium bromide [32]. The synthesis of ultra-small copper nanoparticles was achieved by reduction of copper ions with $NaBH_4$ in the presence of surfactant within millifluidic reactor [33].

We report in this paper, the low cost room temperature facile green synthesis of ultra-small copper oxide nanoparticles (UCuONPs) by using aqueous and alcoholic extracts of *Swertia chirayita* for the bio-reduction of copper (I), their physicochemical characterization and antibacterial efficacy.

2 EXPERIMENTAL

2.1 Materials

Whole dried herb of *Swertia chirayita* was purchased from local market of Aligarh city. It was ground to fine powder with the help of pestle and mortar. The herb powder was sieved to remove the coarse particles and stored in an air-tight container. A soxhlet apparatus was used to extract the *Swertia chirayita* powder using water and ethanol as solvents. A charge of 7 gram of herb powder was placed in a thimble and extracted using 200 ml solvent in each case. The extraction was discontinued when the solvent dropping from the thimble became colorless. The extracts were stored at 4°C for the further experimentation. All other reagents are analytical grade and were used as received.

2.2 Preparation of UCuONPs

For the green synthesis of ultra-small copper oxide nanoparticles (UCuONPs), the solutions of ~7 millimoles of $CuNO_3$ in 50 ml each of aqueous and ethanolic plant extracts were

prepared. The $CuNO_3$ was allowed to react with the herb extracts under constant magnetic stirring for 24 h. The reaction mixtures were centrifuged at 10000 rpm and the supernatant solvent containing unreacted herb extract was removed followed by washing of UCuONPs with distilled water, tetrahydrofuran (THF) and then dimethylsulfoxide (DMSO). The purified UCuONPs were dried at 80°C for further studies.

2.3 Physicochemical Characterization

The morphology, structure and chemical composition of UCuONPs were studied by a variety of techniques including the UV–Visible spectrophotometry (T70 PG Instrument limited), TEM (JEM 2100, JEOL, JAPAN), SEM with EDS (JEOL, JSM, 6510-V, (JAPAN), XRD (PROTO AXRD) and FTIR (Thermo Ominic Nicolet Is50).

2.4 Antibacterial Efficacy Studies

Antibacterial efficacy of thus synthesized UCuONPs was determined against *Staphylococcus aureus* (ATCC-6538) *Escherichia coli* (ATCC-8739) and *Salmonella entrica* (MTCC-3858) by well-diffusion method. The culture of pathogens was subcultured in soyabean casien digest medium broth and 8 mm wells were punched into nutrient agar plates for testing nanomaterial efficacy against the pathogens. Each well was sealed with a drop of agar in order to prevent leakage over the glass surface. Using a micropipette, 75 µl of the suspension of nanoparticles was poured into each well of all the plates and kept in BOD for 24 hours and then in an incubator for further growth. The different levels of zones of inhibition were measured while dimethylsulfoxide blank was used as a negative control and antibiotics tetracycline and ciplox were used as the positive control.

3. RESULTS AND DISCUSSION

3.1 Preparation of UCuONPs

The color of the solutions change with the progress of the reduction of Cu^+ ions to Cu(I) by the aqueous and alcoholic extracts of *Swertia chirayita* with time as shown in the **Fig.1**(**a** & **b**).

Fig.1 The change observed in the color of the solutions with the progress of the reduction of Cu^+ ions by the (a) aqueous extract and (b) alcoholic extract of *Swertia chirayita* with time.

3.2. UV-Vis Studies

The samples of both types of UCuONPs were dispersed in DMSO and sonicated for uniform dispersion of nanoparticles in the dispersing medium. The small aliquots of diluted sonicated UCuONPs samples were used for detection of surface plasmon resonance property (SPR) of copper nanoparticles in the range of 350-800 nm. The absence of any sharp peak in the spectra of both the samples may be attributed to copper nanoparticles having diameters less than 5 nm. Ultra-small nanoparticles do not give any peak in the visible range because of their extremely small size. However, large sized copper nanoparticles show the peak at 560-570 nm. Copper nanoparticles show a wide range of intensities at various wavelengths depending on their size [34-38]. UV-visible spectra of ultra-small copper oxide nanoparticles (UCuONPs) prepared by (a) aqueous and (b) alcoholic extracts of *Swertia chirayita* are shown in the **Fig.2**(**a** & **b**).

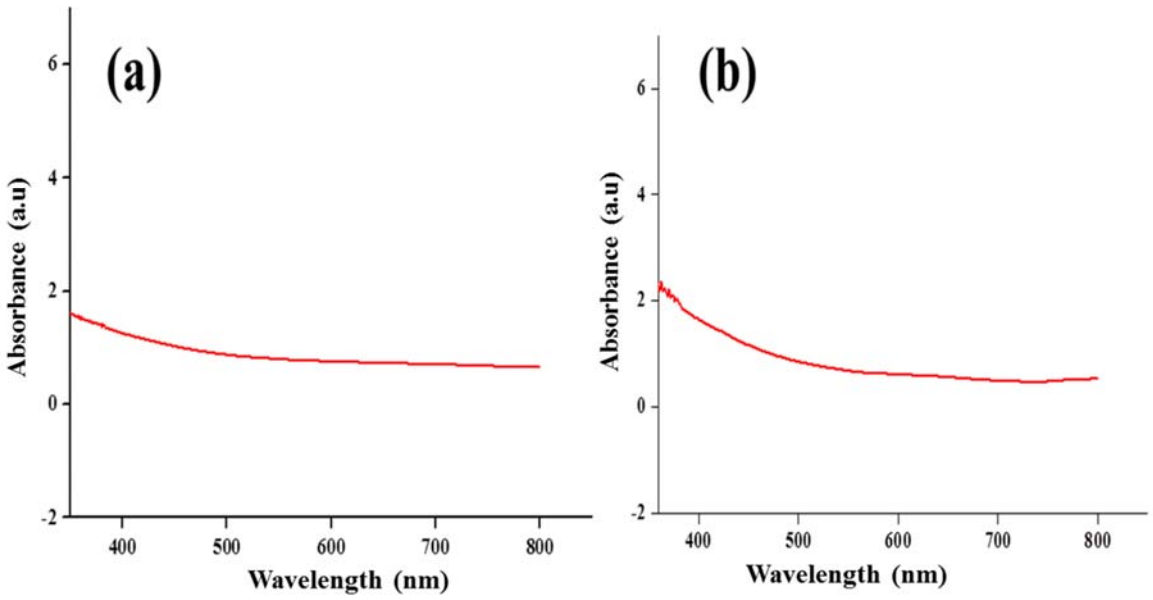

Fig.2 UV-visible spectra of ultra-small copper oxide nanoparticles (UCuONPs) prepared by (a) aqueous and (b) alcoholic extracts of *Swertia chirayita*.

3.3. Scanning Electron Micrographic (SEM) and Energy-Dispersive X-Ray Spectroscopic (EDX) Studies

The SEM micrographs of ultra-small copper oxide nanoparticles (UCuONPs) prepared using aqueous and alcoholic extracts are shown in **Fig.3**(**a** & **b**) and **Fig.3**(**c** & **d**) respectively. Micrographs of thus prepared UCuONPs are suggestive of spherical nanoparticles agglomerated due to binding of the plant extracts.

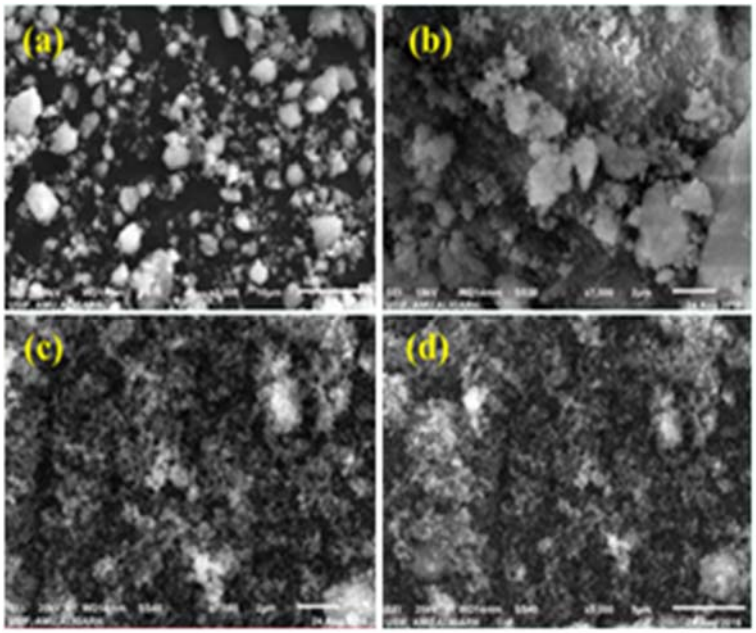

Fig.3 SEM images of UCuONPs prepared by (a) aqueous and (b) alcoholic extracts in two different magnifications.

The EDX spectra of UCuONPs prepared by aqueous and alcoholic extracts showing copper and oxygen signals are shown in **Fig.4**(**a** & **b**). The presence of peaks corresponding to copper and oxygen confirms the presence of copper oxide nanoparticles in both the extracts. The

peak of carbon is also observed in both the spectra that may be attributed to the organics present on the surface of the UCuONPs or to carbon tape used as attachment base.

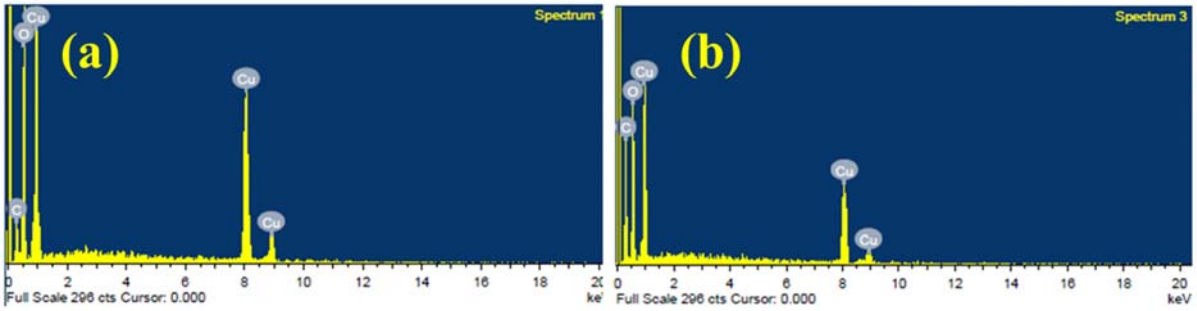

Fig.4 The EDX spectra of UCuONPs prepared by (a) aqueous and (b) alcoholic extracts showing copper and oxygen signals.

3.4. Transmission Electron Microscopy (TEM) Studies

TEM images of thus prepared UCuONPs using *Swertia chirayita* aqueous and alcoholic extracts are shown in **Fig.5(a & b)** and **Fig.5(c & d)** respectively**.** The particles were found to be in the range of 2 to 10 nm of average size and are spherical in shape. The size and shape of prepared UCuONPs generally depend on the antioxidant capacity as well as the capping capability of the plant extract and reaction conditions used in the preparation. The inset shows the SAED pattern confirming the crystalline nature of UCuONPs prepared from aqueous and alcoholic extracts.

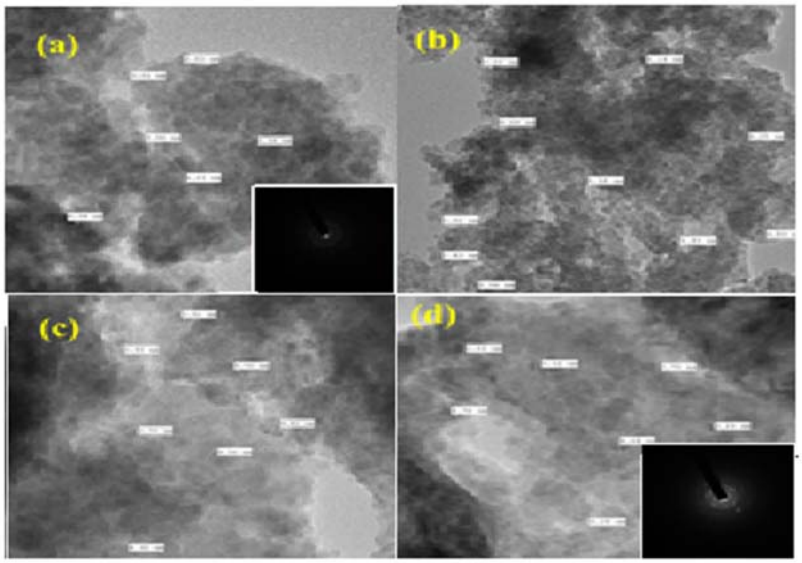

Fig.5 TEM images of thus prepared UCuONPs using *Swertia chirayita* (a & b) aqueous and (c & d) alcoholic extracts in different magnifications.

3.5 FT-IR Spectroscopic Studies

FTIR was used to identify the functional groups of the extract adsorbed on the UCuONPs. **Fig.6(a & b)** shows the FTIR spectra of ultra-small copper oxide nanoparticles (UCuONPs) prepared by aqueous and alcoholic extracts of *Swertia chirayita* respectively. Four bands are common to both the spectra which appear at ~710 cm^{-1}, ~1380 cm^{-1}, ~1630 cm^{-1} and ~3430 cm^{-1} respectively. The broad band near 3430 cm^{-1} is likely due to alcohol/phenol O-H stretch from hydroxyl group expected to be present in the extract such as polyphenols [39]. The absorptions bands observed around 1630 cm^{-1} may be attributed to C=C stretch from the alkenyl group [40]. The absorption peak at 1380 cm^{-1} represents C-F stretching vibration indicating the presence of antioxidants like

polyphenol, tannins, terpenoids etc. A small band observed at ~710 cm^{-1} is likely due to the C-H bending. The different bands appearing above suggest the presence of polyphenols and other biomolecules present in the plant extracts and adsorbed to the UCuONPs even after washing with the different solvents.

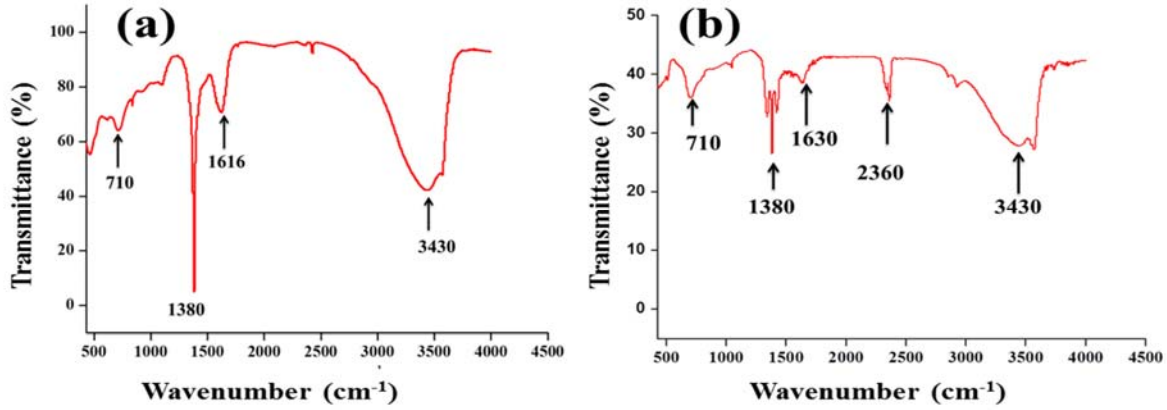

Fig.6 The FTIR spectra of UCuONPs prepared by (a) aqueous and (b) alcoholic extracts of *Swertia chirayita.*

3.6 X-Ray Diffraction (XRD) Studies

The morphological nature of UCuONPs was observed by XRD. **Fig.7**(**a** & **b**) show the XRD spectra of the ultra-small copper oxide nanoparticles (UCuONPs) prepared by aqueous and alcoholic extracts of *Swertia chirayita* respectively. Both the spectra have some common peaks at 2θ = 12º, 25º, 33º, 36º, 39º, 52º. The presence of sharp peaks in the X-ray diffraction patterns confirm that UCuONPs formed by the reduction of Cu^{+} ions by *Swertia chirayita* leaves extracts are crystalline in nature. The diffraction peaks positioned at 2θ values 36°, 39º and 52º are corresponding to the (111), (111) and (113) planes of the crystalline phase of cupric oxide (CuO) respectively. The peaks positioned at 2θ values of 12° and 25° are assigned to the (110) and (111) planes of the crystalline phase of cuprous oxide (Cu_2O) respectively. The presence of peaks corresponding to CuO as well as Cu_2O in the XRD spectra is suggestive of the presence of both the oxides in UCuONPs crystalline phase [41].

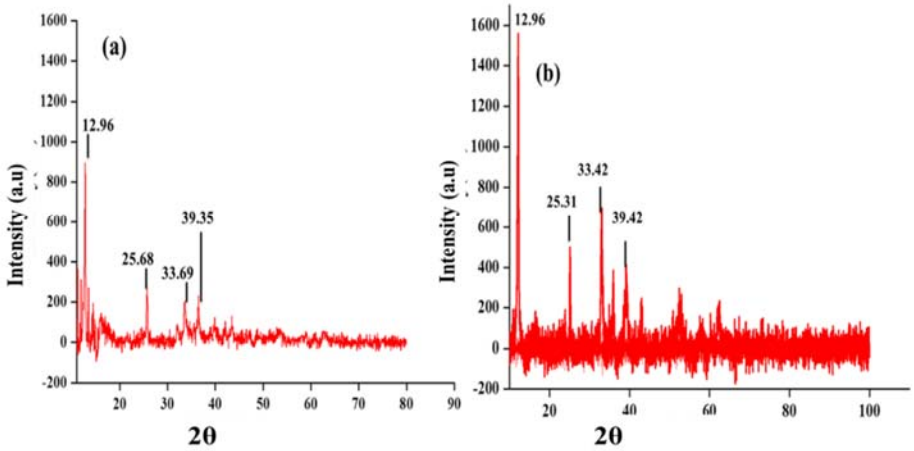

Fig.7 X-ray diffraction spectra of UCuONPs prepared by (a) aqueous and (b) alcoholic extracts of *Swertia chirayita.*

3.7 Antibacterial Studies

Antibacterial activity results revealed that both aqueous and alcoholic extracts of *Swertia chirayita* used for the preparation of UCuONPs acted as effective antibacterial agents against the *Staphylococcus aureus* (ATCC 6538),

Escherichia coli (ATCC-8739) and *Salmonella enterica* (MTCC-3858). It is clear from the transmission electron microscope results that the size of copper oxide nanoparticles is in the range of 2 nm and 10 nm. Both the UCuONPs were dissolved in DMSO (75µL) and their formulation was prepared by mixing equal volumes of both the solutions for testing their antibacterial efficacy. DMSO was used as negative control while tetracycline (5µg/50mL) and ciplox (20 µg/50mL) were used as positive control. **Table-I** and **Fig.6** give the size of zones of inhibition (mm) of two extracts and their formulation against Gram-positive *(Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella enterica*) bacterial strains. The UCuONPs prepared by aqueous extract, alcoholic extract and their 1:1 formulation exhibited the zones of inhibition of 16 mm, 16 mm and 18 mm respectively against *Staphylococcus aureus*. The UCuONPs prepared by aqueous extract, alcoholic extract and their 1:1 formulation exhibited the zones of inhibition of 26 mm, 26 mm and 25 mm respectively against *Escherichia coli*. The UCuONPs prepared by aqueous extract, alcoholic extract and their 1:1 formulation exhibited the zones of inhibition of 25 mm, 24 mm and 23 mm respectively against *Salmonella enterica*. The antibacterial efficacy of the UCuONPs prepared by aqueous extract, alcoholic extract and their 1:1 formulation against *Escherichia coli* and *Salmonella enterica* is fairly same but higher than that against *Staphylococcus aureus*. It may be noted that no synergistic or antisynergistic effect in antibacterial efficacy was observed in the formulations due to similarity in the two types of UCuONPs. It may also be emphasized that UCuONPs have 36% more bactericidal efficacy against Gram-negative bacterial strains as compared to Gram-positive bacterial strain. **Fig.7** shows the bar graph of zones of inhibition of UCuONPs against various pathogens. **Fig.8** zones of inhibition of UCuONPs and their formulations against *Staphylococcus aureus, Salmonella entrica* and *Escherichia coli*. Overall, our observation revealed that UCuONPs are more effective to Gram-negative bacteria as compared to Gram-positive. Thus, the present work has shown UCuONPs to possess effective antibacterial behavior. However, it is important to know the exact mechanism of antibacterial activity and cytotoxicity to mammalian cells. Beside these nanoparticles may have the wide range of contribution if assess to their size, shape, morphology on cytotoxicity are studied. The formulation of UCuONPs using both extracts was also studied and it was observed that the formulations showed similar antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Salmonella entrica*.

Sample (Treatment: 75µl)	**Zone of Inhibition (mm)**		
	Gram-Positive	**Gram-Negative**	
	Staphylococcus aureus	***Salmonella enterica***	***Escherichia coli***
Tetracycline (Positive Control)	40	27	No Activity
Ciplox (Positive Control)	34	40	31
UCuONPs (Aqueous Extract)	16	25	26
UCuONPs (Alcoholic Extract)	16	24	26
1:1 (v/v) Formulation	18	23	25
DMSO (Negative Control)	No Activity	No Activity	No Activity
Concentration of UCuONPs Solution = 0.1g /mL Concentration of Tetracycline Solution = 5µg/50mL Concentration of Ciplox Solution = 20 µg/50mL			

Table-I: Antibacterial efficacy of UCuONPs against Gram-positive and Gram-negative bacterial strains using with tetracycline and ciplox as positive control and DMSO as negative control.

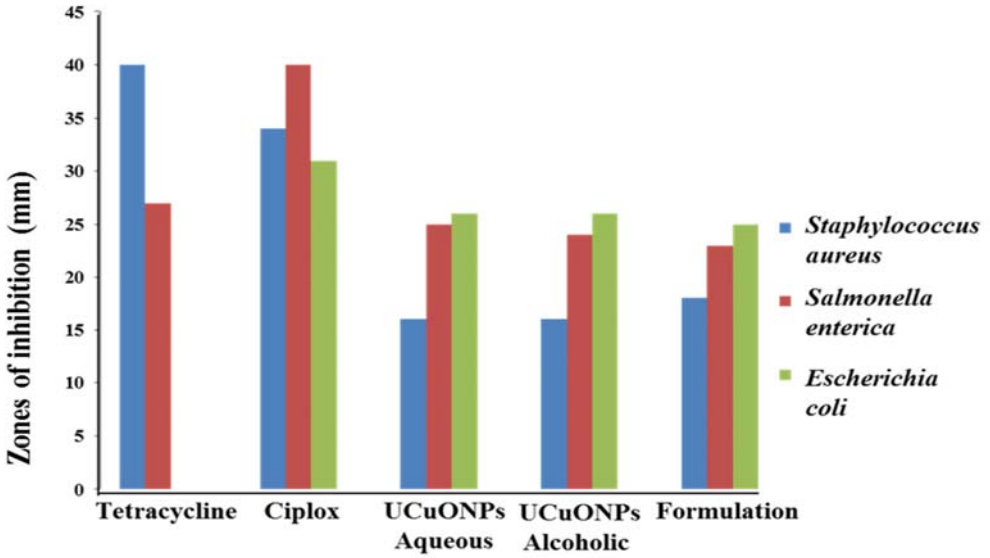

Fig.7 Zones of inhibition of UCuONPs against bacterial strains and positive controls.

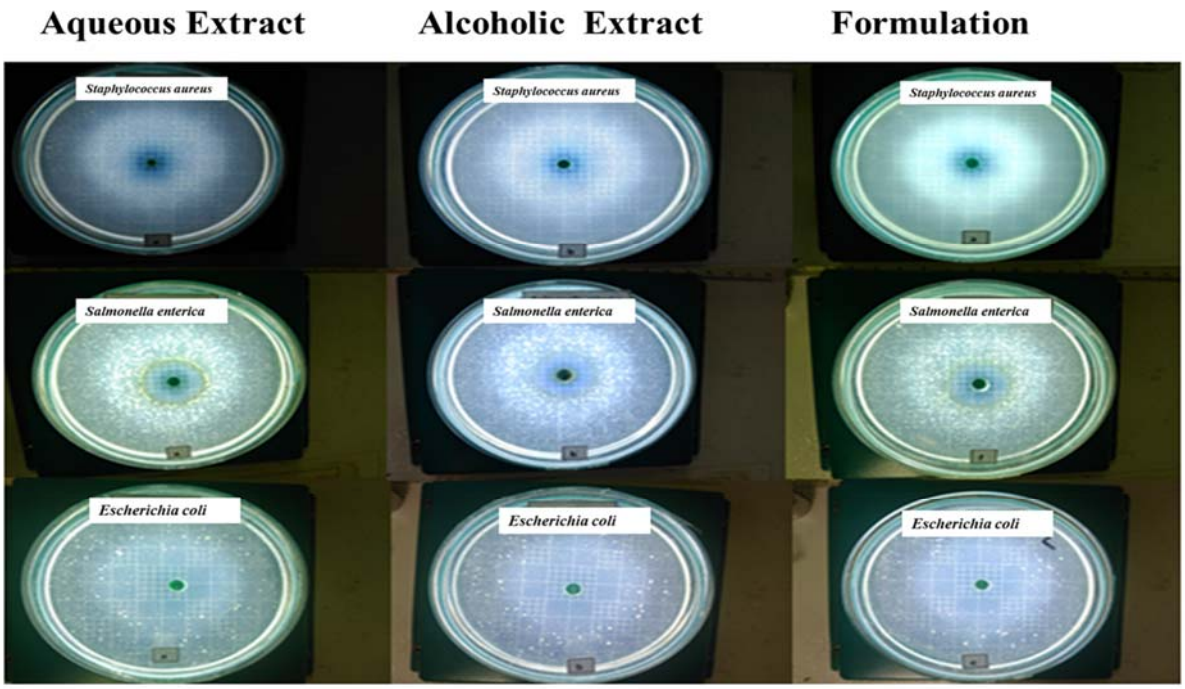

Fig.8 Zone of inhibition of UCuONPs and their formulation against *Staphylococcus aureus*, *Salmonella entrica* and *Escherichia coli.*

4. CONCLUSIONS

The ultra-small copper oxide nanoparticles (UCuONPs) were prepared using aqueous and alcoholic extracts of *Swertia chirayita* in view of the significance of green synthesis nowadays. *Swertia chirayita* has very efficiently reduced the copper (I) ions to UCuONPs. Thus prepared UCuONPs have successfully been characterized by UV-Visible, SEM, TEM, EDX and XRD techniques. The UCuONPs are crystalline in nature and their size ranges between 2-10 nm. The antibacterial efficacy of UCuONPs of Gram-negative (*Salmonella enterica* and *Escherichia coli*) bacterial strains were observed to be better that against Gram-positive (*Staphylococcus aureus*) bacterial strain. The results strongly suggest this approach could be utilized in making possible alternate antimicrobials against resistant bacterial infections by green synthesis.

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