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Research Article

Green Synthesis and Characterization of Cu and Cu_2O Nanoparticles and its Biological Activities Using *Urginea indica*

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Abstract: In the past decade, the development of metal nanoparticles in an eco-friendly manner has become an important aspect in the field of nanotechnology. Our present study reports the utilization of *Urginea indica* (*U. indica*) bulbs for the synthesis of copper (Cu) and copper oxide nanoparticles (Cu_2O NPs). Cytotoxic activity against colon (HCT-116) carcinoma cell lines as well as their antibacterial and scavenging potential were determined. The green synthesized copper nanoparticles were confirmed using various analytical characterization techniques. The cytotoxic effect of the NPs was studied by MTT assay, and scavenging efficacy was assessed by DPPH and ABTS assays. Furthermore, green synthesized nanoparticles were evaluated for their antibacterial activity by the Agar disc diffusion method against multidrug-resistant bacterial strains. The nanoparticles were observed to be almost spherical in shape with an average diameter of 66.99nm as assessed by SEM analysis. Nanoparticles revealed dose-dependent antioxidant activity and antimicrobial activity against bacterial strains *viz. Enterococcus faecalis, Bacillus cereus*, *Escherichia coli*, *Serratia marcescens,* and also *in vitro* studies revealed dose-dependent cytotoxic effects treated against HCT-116 cell line with IC_{50} of 56.726 µg/mL. The data obtained in the study shows that Cu-Cu_2O NPs obtained from *U. indica* bulbs have potent therapeutic value in the pharmaceutical industry.

Key words: *Urginea indica* bulbs, Green Synthesis, Copper Nanoparticles, Antioxidant, Antimicrobial, Cytotoxicity.

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1. INTRODUCTION

In recent times, nanotechnology has shown tremendous progress in terms of scientific research and applications. The vast potential of nanotechnology finds large biomedical applications such as gene delivery, biosensors, drug, cancer treatments, and diagnostic tool[1]. The manufacture of nanoparticles by eco-friendly green nanotechnology method has gained enormous interest worldwide. The metal-based nanoparticles with unique physicochemical properties have been considered a challenging alternative medicine for various disease treatments. Several studies revealed that the most frequently used metal salts are iron, silver, copper, gold, platinum, palladium, cadmium, titanium, zinc, magnesium, etc[2], which are widely used for antimicrobial activities, drug delivery, bio fabrics, and cancer therapy [3]due to its low cost, availability and greater stability. In ancient times, copper was used in wound healing and also as an antimicrobial agent [4,5]. During the Second World War, soldiers from Japan added a bit of copper into the drinking water bottles to avoid dysentery[6]. Nanoparticles can be synthesized using both chemical and physical methods but these methods are expensive, have high-energy consumption, comparatively have low production efficiency and also cause various health issues [7]. The green-reduction method is more suitable for synthesizing inorganic NPs by completing chemical reduction using biological sources[8]. The production involves the reduction reaction between the metallic ion precursor and plant extract. There is a huge demand for green synthesis due to its sustainability, reliability, and eco-friendly, and also due to the presence of phytochemicals. The major advantage of the green synthesis of nanoparticles is, readily biodegradable and many studies advised that they can be used efficiently in environmental and biomedical applications. *U. indica* commonly known as Indian squill is a perennial bulbous plant belonging to the family Hyacinthaceae. It is distributed in India, Africa, and Mediterranean regions, and grows on sandy grounds and sloping hills. It occurs in the southern and peninsular parts of India including coastal and temperate regions of the Himalayas[9]. *U. indica* bulbs have been used as a source of medicine from ancient times. The important bioactive compounds present in *U. indica* bulbs are polyphenols, carotenoids, flavonoids, anthocyanidins, terpenoids, and omega fatty acids, these compounds play significant pharmacological effects in human health as an anti-inflammatory, antioxidant, antimicrobial, antiallergic, antidiabetic and anticancer agent, treating asthma, rheumatism, and dropsy[10, 11]. The present study was carried out for the first time on the synthesis of copper nanoparticles from methanolic extract of *U. indica*, a highly important medicinal plant. Herein we have focused on evaluating the pharmacological applications namely antioxidant, antimicrobial and cytotoxicity properties of the synthesized nanoparticles.

2. MATERIAL AND METHODS

2.1 Chemicals

Copper sulphate ($CuSO_4$), Methanol, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis 3 ethylbenzthiazoline-6-sulfonic acid (ABTS), Potassium persulfate, Muller Hinton agar, dimethyl sulfoxide(DMSO), ascorbic acid, meththiazolyl diphenylnyl-tetrazolium bromide(MTT), Dulbecco's Modified Eagle's Medium (DMEM), 10% FBS a deionizedsed water used in this investigation were acquired from Hi-media laboratories (Bengaluru, India). All other reagents were analytical grade and obtained from commercial sources.

2.2 Experimental microorganisms and cell lines

The microbial strains were obtained from the Department of Microbiology, Bangalore University, Bengaluru. The bacterial strains included two Gram-positive *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (MTTC 497), and two Gram-negative *Escherichia coli* (MTCC 443), *Serratia marcescens* (NCIM 291). Fungal strains *Aspergillus niger* (MTCC 404), *Trichoderma harzianum* (MTCC 5179) were obtained from MTCC, IMTECH, Chandigarh. HCT-116colon carcinoma cell lines were procured from National Centre of Cell Science (NCCS), Pune.

2.3 Preparation of plant sample

Fresh bulbs of *U. indica* were collected from different regions of Karnataka during the Monsoon season. Bulbs were washed, air-dried separately in shade, coarsely powdered, and stored in the neat labeled airtight container until further use. The cold percolation method was used for the preparation of bulb extract using methanol as per the method described by Rosenthaler[12]. The selected plant *Urginea indica* was authenticated by Dr.V.Rama Rao, Research officer, Central Ayurveda Research Institute (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India Reference number SMPU/CARI/BNG/2022-23/Drimia indica or *Urginea indica* belongs to Hyacinthaceae (RRCBI-20160)

2.4 Phytochemical screening of U. indica

The qualitative phytochemical screening was performed as per the standard protocols to identify different classes of compounds (phenols, saponins, terpenoids, alkaloids, flavonoids, glycosides, tannins, proteins, amino acids, and carbohydrates) present in the plant extract[13].

2.5 Phytochemical quantification of U. indica

2.5.1 Total Phenol content (TPC)

The total phenolic content in methanolic extract of *U. indica* bulb was determined with Folin-ciocalteu reagent spectrophotometrically using standard gallic acid curve described by Hossain13.1mL (1 mg/ mL) of *U. indica* bulb extract was mixed with 0.2 mL of Folin-Ciocalteu reagent and incubated for 5 min at RT, followed by 2mL of 7% Na_2CO_3 solution and later it was incubated for 90 min in dark. Absorbance was noted at 725 nm. The assay was performed in triplicates and the mean was calculated along with the standard deviation.

2.5.2 Total Flavonoid content (TFC)

The TFC of methanolic extract of *U. indica* bulbs was determined spectrophotometrically a using standard quercetin curve, with slight modifications as described by Hossain et al., 2013. 13. For TFC, 1mL (1 mg/ mL) of *U. indica* bulb extract was mixed with 0.3 mL of 5% $NaNO_2$ solution, followed by incubation for 5 min at RT. Then 0.3mL of 10% $AlCl_3$ solution was added and the contents were incubated for 6 min at RT. Finally, 1mL of NaOH (1M) was added and absorbance was noted at 510 nm. The assay was performed in triplicates and the mean was calculated along with the standard deviation.

2.5.3 Total tannin content (TTC)

The TTC of the methanolic extract of *U. indica* bulbs was determined spectrophotometrically using the procedure described by Kathirvel et al.[14] In this procedure, 4 mL of bulb extract was mixed with 3 mL of $FeCl_3$ solution (0.1 M in 0.1 M HCl), followed by the addition of 3 mL of potassium ferricyanide (0.008 M). The reaction mixture was allowed to stand at RT for 15min. Absorbance was noted at 720 nm. Tannic acid was taken as a standard and from the plot of absorbance vs. concentration, the TTC was determined and the value was expressed in milligrams of tannic acid equivalents (TAE) per gram dry weight of *U. indica* bulb extract, in mean value ± standard deviation (n=3).

2.6 Green synthesis of copper nanoparticles

Cu-Cu_2O NPs were synthesized by reduction method.10 mM solution of copper sulphate was prepared double-distilled water. Plant extract of 10 mg/mL concentration was prepared in methanol. Both the above solutions were mixed in equal volumes and initial color chang, and pH change were recorded. Lambda max wavelength was recorded by UV Spectrophotometer and presence of nanoparticles was confirmed. The solution containing the desired nanoparticles was filtered by a 0.22 micron syringe filter. The filtered solution was then dried and powdered. The purified nanoparticles were used for further characterization and various other biological activities [15].

2.7 Characterization of copper nanoparticles

The preliminary characterization of Cu-Cu_2O NPs was carried out using a UV-vis spectrophotometer (UV-1800 SHIMADZU) working on scanning range of 200-800 nm[10]. Methanol was used as a blank. Identification of the crystalline phase of the synthesized Cu-Cu_2O NPs was performed using an advanced power X-ray diffractometer (XRD, Siemens' D5005). The crystallite size of the NPs was found using the Scherer's formula, $d= K\lambda/\beta \cos\theta$, where d is the crystallite size, λ is the wavelength (λ= 1.5406 A°), K is the Scherer's constant (K= 0.9), β is the half-width maximum and θ is the diffraction angle [16]. FTIR measurements were used to identify the possible functional groups associated with Cu-Cu_2O NPs formation. The infrared spectrum of the sample was measured with KBr disc in the wavelength range of 4000-512 cm^{-1} using Perkin Elmer FTIR Spectrophotometer [17]. The morphology and elemental composition of Cu-Cu_2O NPs were determined by scanning electron microscope (TESCAN VEGA 3) at an accelerating voltage of 0.1 to 30Kv equipped with (EDX) Energy dispersive x-ray spectroscopy [18]. The zeta potential analysis was carried out in Litesizer 500 equipment.

2.8 Antioxidant activity

2.8.1 DPPH assay

The free radical scavenging activity of CuNPs were determined by using the DPPH method. 3 mL of copper nanoparticles solution in methanol was prepared in series at different concentrations (1, 10, 25, 50 and 75 µg/mL). Then 3mL methanolic solution of DPPH was added to each solution of the NPs solution and made up to 6 mL. All the solutions were mixed thoroughly and then allowed to stand in the dark for 30 min at room temperature. Control was prepared without adding CuNPs. The UV-vis spectrum of the colored solution was measured and the absorbance was noted at 517 nm. A reduction in the absorbance intensity was observed. Percentage scavenging was calculated using the following formula [19].

% DPPH scavenging activity= (OD Control –OD Sample/ OD Control) x 100

2.8.2 ABTS assay

The antioxidant activity of the samples was measured by ABTS decolourization assay according to the method followed by Re et al.[20] with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 h at room temperature in the dark. 2mL of CuNPs solution in methanol was prepared in series at different concentrations (1, 10, 25, 50 and 75 µg/mL). Then 2 mL of ABTS was added to each test tube and all the test tubes were thoroughly mixed and kept in dark for 10 min. Then OD values were noted at 734 nm. Percentage scavenging was calculated using the following formula.

% ABTS scavenging activity = (OD Control –OD Sample/ OD Control) x 100

2.9 Antimicrobial activity

2.9.1 Antibacterial activity by Agar disc diffusion method

The antibacterial potential of biosynthesized CuNPs was determined by using agar disc diffusion method as described by Brown et al. [21]. About 20 mL of molten and cooled nutrient agar media is poured into sterilized petri plates. The plates were inoculated with previously mentioned organisms. Filter paper discs (6 mm in diameter) were prepared from Whatman No.1 filter paper and were sterilized. Discs containing 10, 20 and 30µg/mL of CuNPs were impregnated on the agar plates. Ciprofloxacin was used as an antibacterial standard and DMSO was used as a negative control against all pathogens. Experiments were performed in triplicates. For diffusing the active compounds in the medium, the plates were kept at 4°C for 2 h. Then the test plates were incubated at 37°C for 24 h. Zones of inhibition of all the samples were measured after 24 h.

2.9.2 Antifungal activity by well diffusion method

The antifungal activity was determined by well diffusion method [22]. About 25mL of potato dextrose agar was poured into a sterile petri plate. The plates were allowed to solidify, after which, 100 µL of fungal spore suspension of mycelia fungal pathogens were swabbed using a sterile cotton swab on the plates and wells were made. Test samples of various concentrations such as 10, 20 and 30µg/mL were loaded into the wells and clotrimazole was used as a positive control. All the drug-loaded petri plates were incubated at 37°C for 72 h. The antifungal activity was determined by measuring the zone of inhibition around the wells.

2.10 Cytotoxicity of green synthesized CuNPs

To determine the anticancer activity of CuNPs and *U. indica* bulb extract, cell viability study was done with the MTT reduction assay [23]. Colon carcinoma (HCT-116) cell lines were obtained in high glucose Dulbecco's Modified Eagle's medium

(DMEM), enhanced with fetal bovine serum 10% in a humidified incubator at 37°C and 5% CO_2. The cell lines were seeded in a 96-well plate for 24 h, in 200 µl of DMEM with 10% FBS. After that, the media was removed and replaced with a suspension of various concentrations of Cu-Cu_2O NPs(12.5, 25, 50, 100 and 200µg/mL)and the cells were incubated for 48 h. Then MTT was added and incubated at 37°C for another 4 h. The medium was then removed and 200 µL of DMSO was added to each well. OD value was subjected to calculate the percentage of viability by using the following formula.

Percentage of cell viability = (OD Control –OD Sample/ OD Control) x 100

3. STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (SD). All the experiments were repeated thrice, and data were (SD). All the experiments were repeated thrice, and data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. $p<0.05$ was considered statistically significant.

4. RESULTS AND DISCUSSION

4.1. *Phytochemical screening of U. indica*

The preliminary phytochemical screening of methanolic bulb extract of *U. indica* were evaluated and the results are shown in the (Table 1). *U. indica* extract showed a high content of flavonoids, tannins, alkaloids, saponins and phenolic compounds. These above-mentioned compounds show many biological activities and therapeutic values including antimicrobial, antioxidant and anticancer activity.

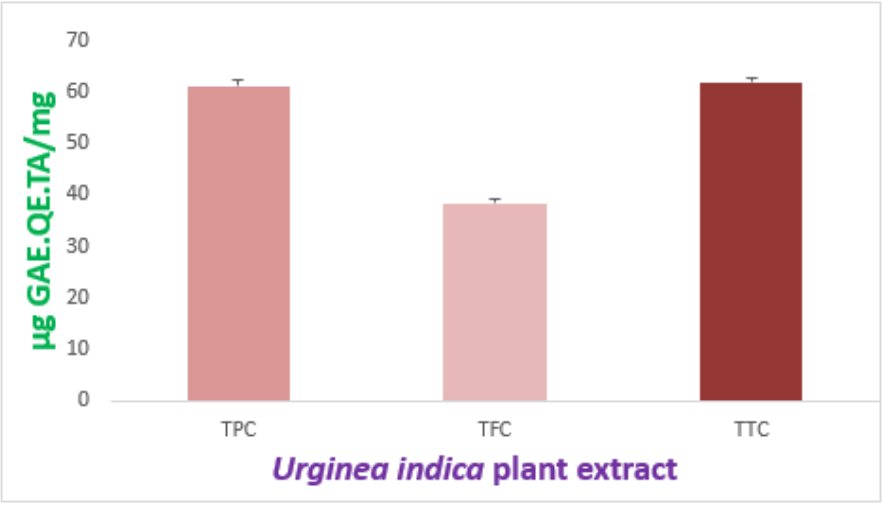
Table 1: Preliminary phytochemical screening of *U. indica*

Phytochemicals	Results +: Present
Phenols	+
Saponins	+
Terpenoids	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Tannic acid	+
Proteins & amino acids	+
Carbohydrates	+

'+' indicates that the compound is present

4.2. *Quantification of phytochemicals in U. indica bulb extract*

Phytochemicals which are present in the plant materials like phenols, terpenoids, flavonoids, tannins and amino acids and are known as excellent bio-substituents to dangerous reducing, stabilizing or capping agents. The presence of these active phyto compounds clearly proved that, the bulb extract has effective antioxidant properties and further as a source of exploration in the pharmaceutical industry. To determine the presence of these phytochemicals in methanolic extract, various assays were conducted. The analysis revealed the presence of 61.23 µg/mg TPC in GAE, 38.46 µg/mg TFC in QE and 62.01 µg/mg TTC in TA shown in (Fig.1). Thus, these assays revealed the presence of phenols, flavonoids and tannins in the methanolic extract of *U. indica.* Similar work was conducted earlier in *U. indica*, where the phenol content in 1g methanolic extract of *U. indica* was found to be 6.27mg GAE/g, total flavonoid in 2mg/mL methanolic plant extract was found to be 13.66 mg/g quercetin equivalent, the tannin content in 0.1g crude extract was found to be 130.10 mg GAE/g [24].



values are presented as mean ±Standard error from triplicate investigation.

Fig.1: Total phenolic (µ g GAE/mg), flavonoid content (µ g QE/mg) and tannic acid content (µ g TA/mg) determination in the extract of *U. indica*.

4.3. *Formation of nanoparticles*

In the present study, Cu-Cu_2O NPs were successfully synthesized in a simple and eco-friendly manner by reducing copper ions present in the methanolic extract of *U. indica.* The formation of Cu-Cu_2O NPs was primarily detected by visualizing the change in color from colorless to dark green, indicating the formation of Cu-Cu_2O NPs as shown in the (Fig.2). Many parameters including temperature, pH, number of phytochemicals present in the plant, and the concentration of metal ions influence the reduction process.

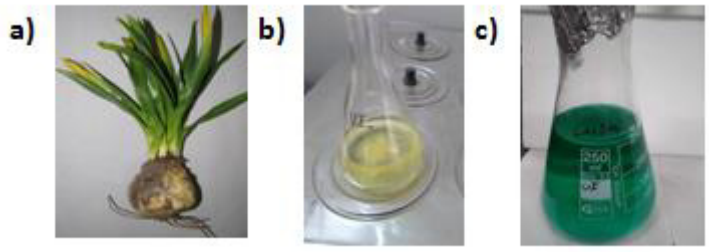


Fig.2 :(a) *U. indica* bulb(b) *U. indica* bulb extract (c) *U. indica*Cu-Cu_2O NPs

4.4. *Characterization of nanoparticles*

The bio-reduction of copper ions in the extract was observed by UV-visible spectra given in the (Fig.3). The size, phase identification and crystalline nature of synthesized NPs were determined by XRD analysis. The XRD pattern of the NPs contains both the characteristic peaks of both copper and copper oxide. The diffraction pattern of synthesized NPs showed diffraction peaks at around 29.1, 36.4, 42.2, 61.4 and 73.5° 2θ values which are indexed to the crystal planes (110), (111), (200), (220), and (311), respectively depicted in (Fig.4). The peak positions are consistent with the standard document of Cu_2O (JCPDS No. 05-0667). The XRD pattern revealed that synthesized nanoparticles were a mixture of metallic Cu and copper (I) oxide (Cu_2O). Mustafa Bicer and Ilkay Sismanin[25]reported that the characteristic peak of Cu_2O emerged only by decreasing the reaction temperature from 85°C to 60°C, since at 60°C reducing agents could not completely reduce Cu^{2+}into Cu^0 atoms. SEM analysis was done to visualize the surface morphology, it showed an average particle size of 66.99 nm shown in (Fig.5). As it is evident from the figure, NPs are spherical in shape and the agglomerations might be due to the adhesive nature of the samples. Similar morphology was observed for Cu-based NPs when synthesized using *Acalypha indica* and *Thymus vulgaris*. Fig.6 shows the EDX spectrum showing elemental composition of prepared Cu-Cu_2O NPs and it also showed other weak signals of oxygen, sulphur, sodium, Carbon, Nitrogen due to the phenolic compounds, flavonoid, carbohydrates and saponins in the bulb extract of *U. indica.* The FTIR spectra obtained is shown in the (Fig.7). The FTIR spectrum revealed the presence of chemical bonds responsible for the formation of Cu-Cu_2O NPs. The FTIR spectrum was usually measured in the range 4000-512 cm^{-1} [26]. The strong peaks at 3677 cm^{-1} represents the presence of OH stretch, ~1400 cm^{-1} corresponds to a carboxylic acid group, the peak at 866 cm^{-1} corresponds to an aromatic group. The Zeta Potential of Cu-Cu_2O NPs is shown in (Fig.8), the zeta potential obtained from the nanoparticles showed a negative surface charge with a value of − 17.5mV. The sample showed a negative value lower than -30 mV. The obtained result suggests good colloidal stability of the Cu-Cu_2O NPs and can be attributed to the high coating content. The solution containing the test sample maintained good stability, as there was no aggregation when kept for an extended period of time [27].

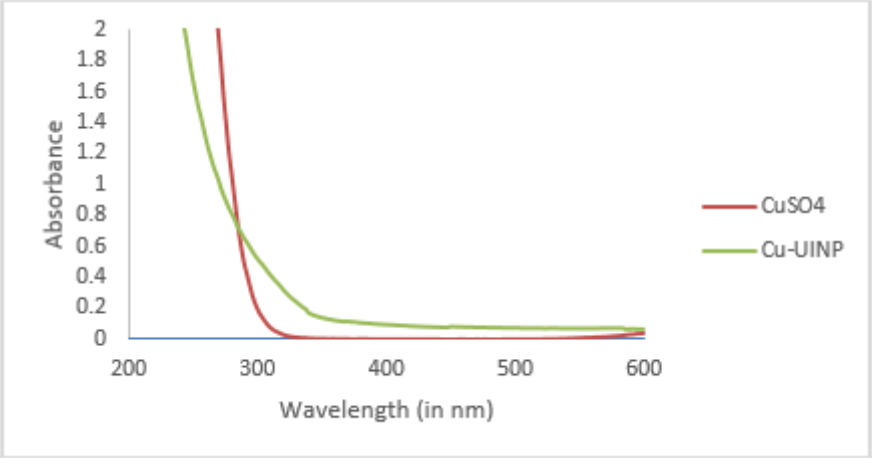


Fig.3: UV-vis absorption spectrum of Cu-Cu_2O NPs green synthesized from bulb extract from *U. indica*

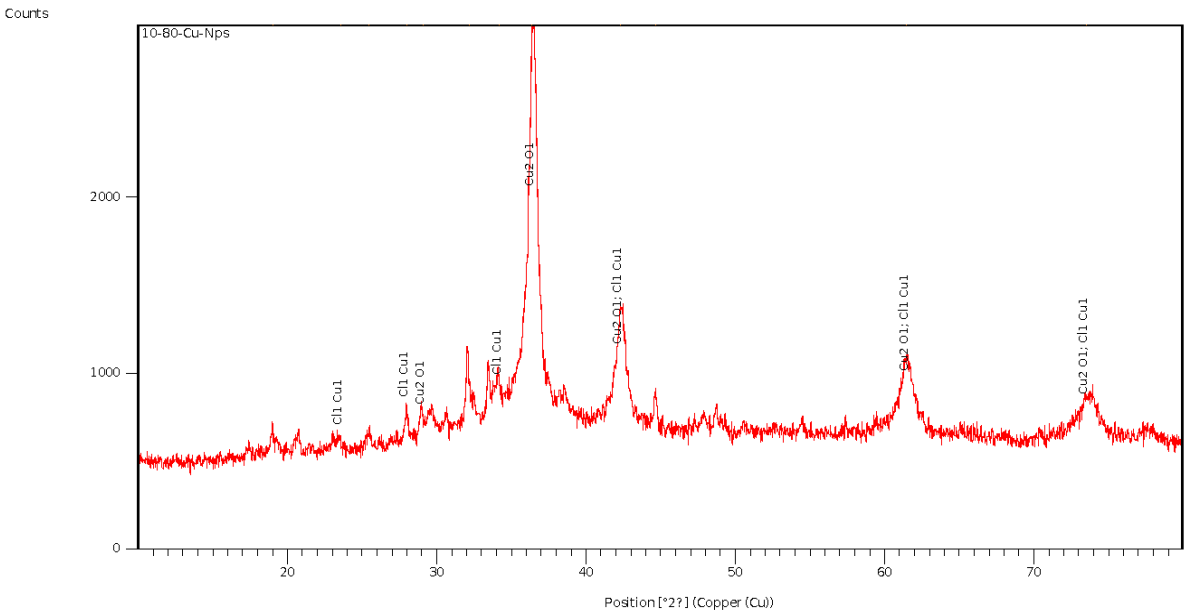


Fig.4: XRD pattern of Cu-Cu_2O NPs green synthesized from bulb extract from *U. indica*

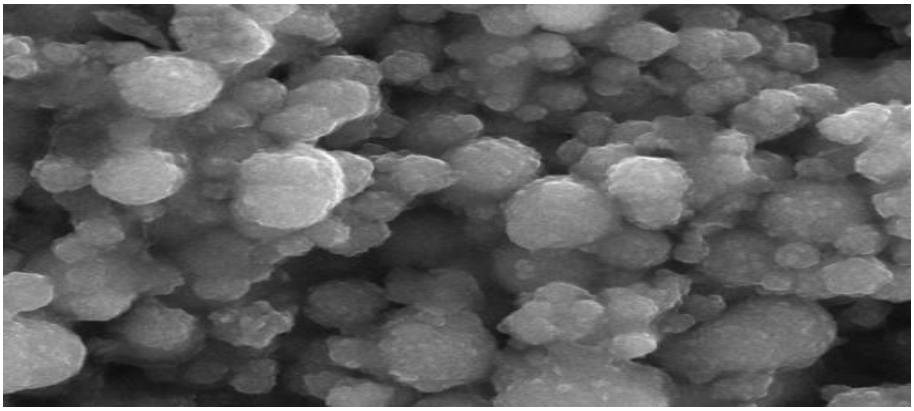


Fig.5: Image of scanning electron microscopic of Cu-Cu_2O NPs green synthesized from bulb extract from *U. indica*

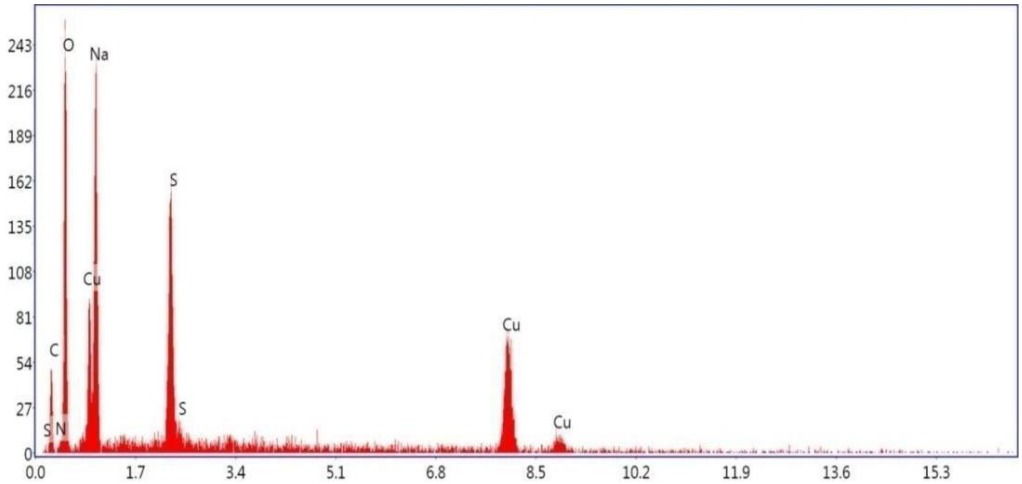


Fig.6: EDX analysis of Cu-Cu_2O NPs green synthesized from bulb extract from *U. indica*

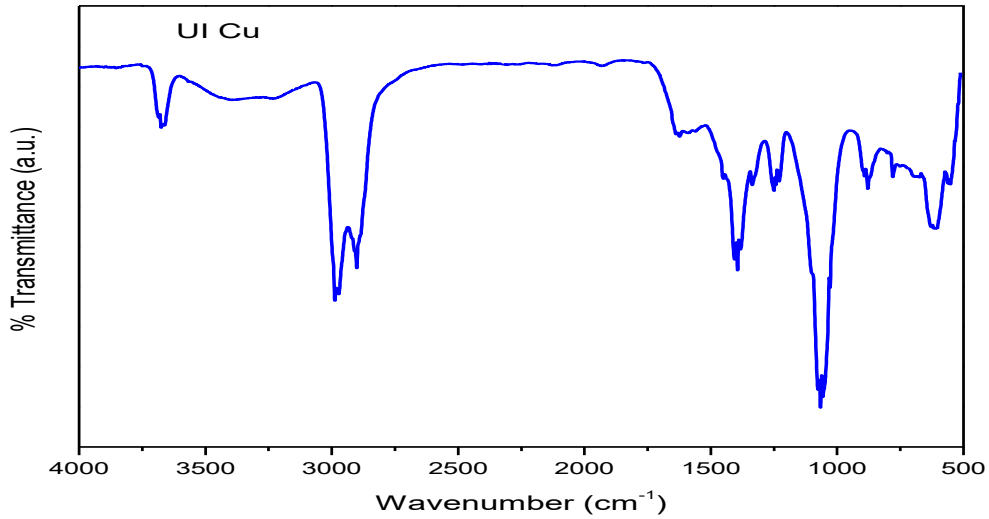


Fig.7: FTIR graph of Cu-Cu_2O NPs green synthesized from bulb extract of *U. indica*

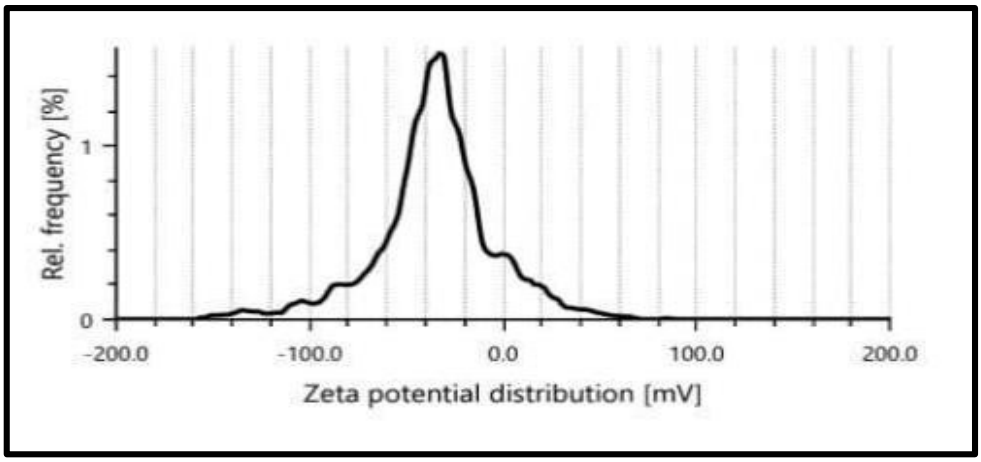


Fig.8. Zeta potential of Cu-Cu_2O NPs green synthesized from bulb extract of *U. indica*

4.5. *Antioxidant activity*

4.5.1. *DPPH assay*

The assay is based on electron transfer; the free radicals are reduced in the presence of the antioxidant and become colorless solutions by gaining electrons [28]. In our study, the DPPH free radical scavenging property of CuNPs showed an IC_{50} value of 82.22 µg/mL. The result showed that, the inhibition percentage substantially increased with the increasing concentration as shown in the (Fig.9). Agreement with our experiment, the previous studies revealed that, the metal nanoparticles had excellent antioxidant activities and they destroyed various free radicals such as DPPH, ABTS and FRAP [29]. Antioxidants have a significant impact on the functions of all biological systems. Due to the interaction of biomolecules with molecular oxygen free radicals are generated [30].

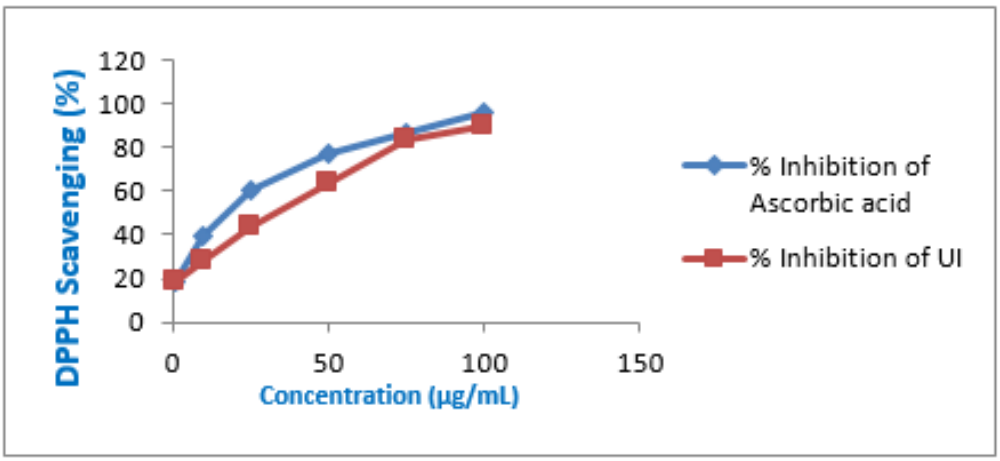


Fig.9: DPPH scavenging activity of CuNPs from bulb extract of *U. indica*

4.5.2. *ABTS assay*

ABTS is an excellent tool which is widely used for detecting hydrogen donating antioxidants. It is one of the frequently used scavenging activities of liquid mixtures, drinks and plant extracts[31].The activity is based on ABTS cation radical formation and dark blue colored ABTS reduced by an antioxidant into colorless solution, which was mentioned by Seong et al[32]. The presence of bioactive compounds in the plant extract that inhibit the potassium persulphate activity may reduce the production of ABTS. The percentage of inhibition showed dose-dependent activity shown in the (Fig.10). The CuNPs were capable of decolorizing the ABTS cation radical and neutralizing free radicals. CuNPs showed good scavenging activity with an IC_{50} value of 50.46 µg/mL.

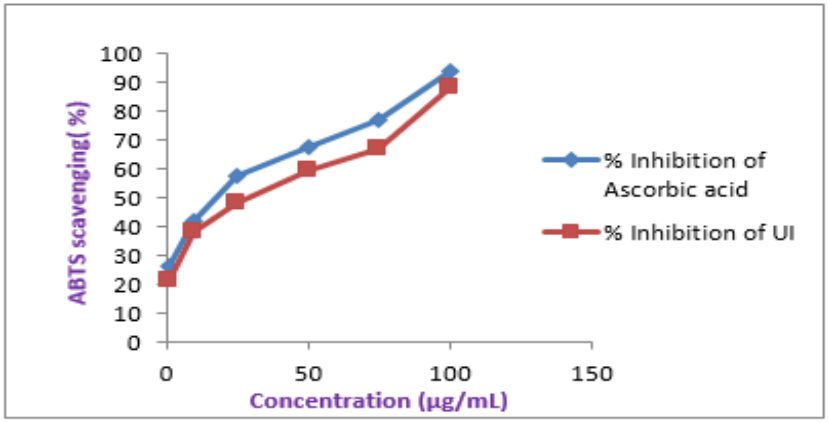


Fig.10: ABTS scavenging activity of Cu NPs from bulb extract of*U. Indica*

4.6. *Antimicrobial activity*

4.6.1. *Antibacterial activity by Agar disc diffusion method*

The results of the antibacterial activity of the CuNPs at different concentrations (10, 20 and 30 µg/ml) against the gram positive (*Enterococcus faecalis* and *Bacillus cereus*) and gram-negative (*Escherichia coli* and *Serratia marcescens*) bacterial strains by disc diffusion method as shown in the (Fig.11). It was found that green synthesized CuNPs showed a considerable antibacterial effect towards Gram-positives than the Gram-negative bacterial strains. This may be due to the cell wall of gram-positive bacteria having a thick peptidoglycan layer while this layer is thin in gram-negative bacteria. In addition, the binding of CuNPs to the proteins in the bacterial cell wall could cause its damage and it is responsible for leakage of cellular contents which ultimately results in the death of the bacterial cell [33]. In copper oxide nanomaterial derived from *M. charantia* the highest efficacy was observed against *Bacillus cereus* with highest zone of inhibition (31.66 mm) [34]. Similarly, *E. coli* was the most susceptible microorganism with a zone of inhibition (28 mm) by copper oxide nanoparticles [35]given in the Table 2.

Table 2: Antimicrobial activity of UI CuNPs against human pathogenic bacteria

Strains	Positive control	Zone of inhibition in mm		
		10µg/mL	20µg/mL	30µg/mL
E. faecalis	23	15	16	13
E. coli	24	12	13	14
S. marcescens	23	12	`13	13
B. cereus	24	11	14	14

All the readings of the triplicates. In all the concentration it had showed inhibition activity

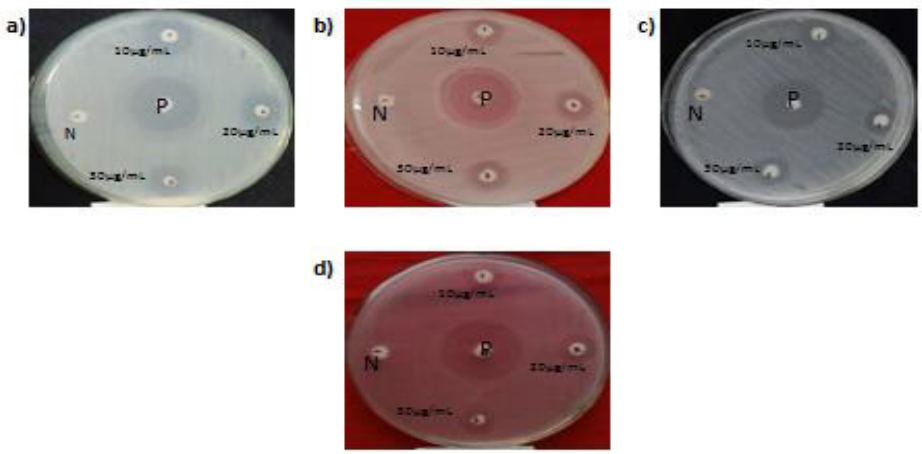


Fig.11: Zone of inhibition with different concentration of *U. indica* CuNPs by Agar disc diffusion method (a) *Enterococcus faecalis*(b) *Escherichia coli* (c) *Serratia marcescens* (d) *Bacillus cereus* (N - Negative & P - Positive)

4.6.2 *Antifungal Activity by well diffusion method*

Antifungal analysis of green synthesized CuNPs was carried out using different concentrations (10, 20 and 30µg/mL) against *Aspergillus niger* and *Trichoderma viride*. Dose-dependent responses were observed against the fungal strains given in Table 3. *A. niger* did not show any activity at 10µg/mL concentration, whereas at 30µg/mL it showed a higher zone of inhibition of 20mm, test samples showed a moderate activity against *T. viride* given in the (Fig.12).

Table 3: Antifungal activity of *U. indica* CuNPs against human pathogenic fungi

Fungal Strains	Positive control	Zone of inhibition in mm		
		10µg/mL	20µg/mL	30µg/mL
A. niger	20	-	11	20
T. viride	18	11	13	15

All the values mentioned in the table are the mean value of the triplicates. '-' indicates no inhibition

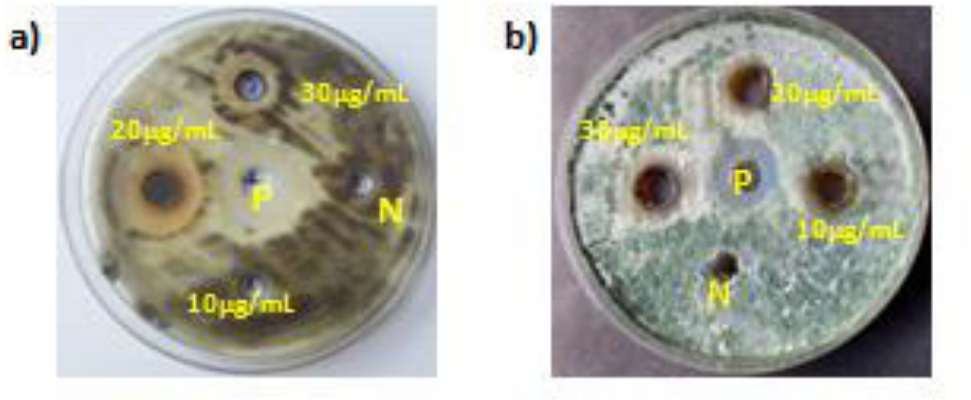


Fig.12: Zone of inhibition with different concentration of *U. indica* CuNPs by well diffusion method (a) *Aspergillus niger*(b) *Trichoderma viride*(N - Negative & P - Positive)

4.7. *Cytotoxicity of green synthesized CuNPs*

In the present study, the green synthesized CuNPs showed cytotoxic activity against colorectal carcinoma cell line (HCT-116) in a dose-dependent manner, while the crude plant extract did not show any activity. Morphological features before and after treatment of HCT cancer cells with CuNPs shown in the (Fig.13). The percentage of cancer cell growth inhibition was found to be high with increasing the concentrations of CuNPs.[36] The Inhibitory Concentration (IC_{50}) of the phyto mediated CuNPs was recorded at 56.726 µg/mL against HCT-116 cell line given in the (Fig.14). Similar work was done in *Ormocarpum cochinchinense* using CuO NPs which showed significant cytotoxicity effect on human colon cancer cell line with IC_{50} value of 4µg/mL[37].Copper oxide nanoparticles using *Acalypha indica* leaf extract incorporated with grapheme oxide showed 70% cytotoxic activity at 100 µg/mL Kavitha et al.[38] The cytotoxic effect of plants is mainly contributed by the presence of secondary metabolites like alkaloid, tannin, terpenoid, glycoside, steroid, and flavonoid in their extract. Previous reports showing that the viability of cancerous and noncancerous cells decreases with increase in the concentration of green synthesized nanoparticles obtained results shows similar findings Aziz et al.[39].

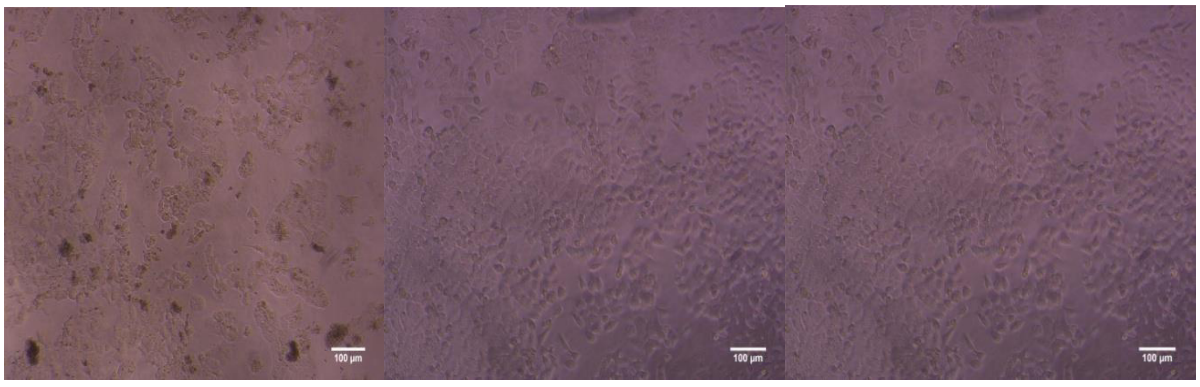


Fig.13: Morphological features before and after treatment of HCT cancer cells with CuNPs (a) UI-CuNPs (b) Untreated (c) Standard(Cisplatin)

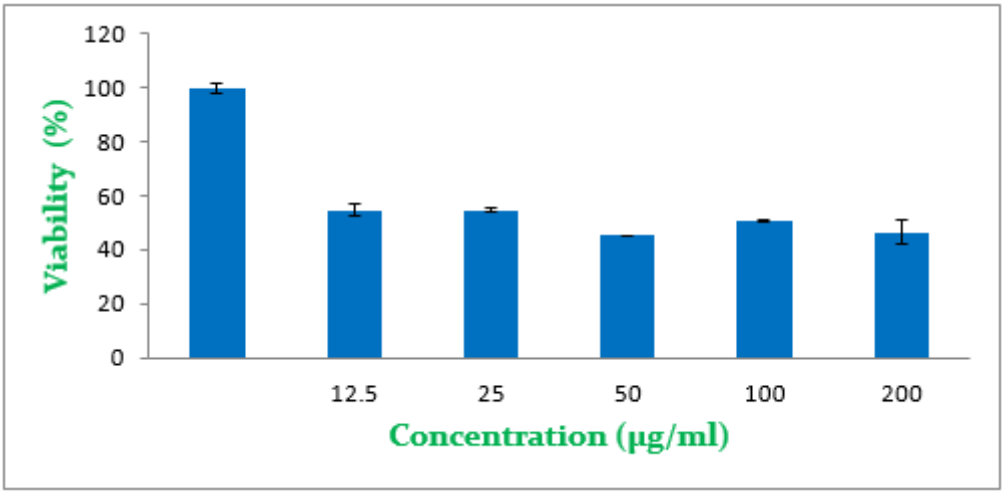


Fig.14: Cytotoxic activity of Cu-Cu_2O NPs from bulb extract of *U. indica* against HCT-116 cell line.

5. CONCLUSIONS

In the present study, a simple, rapid, modest, cost effective, efficient and eco-friendly method for green synthesis of Cu-Cu_2O NPs was obtained using the bulb extract of *U. indica*. The physicochemical properties were characterized by XRD, FTIR, SEM, EDX and Zeta potential techniques. The green synthesized Cu-Cu_2O NPs has the ability to reduce the copper sulphate into copper which is visibly shown by the color change from blue to dark green. The nanoparticles were found to be spherical in shape with an average size of 66.99 nm and the FTIR analysis confirmed the presence of alkaloids, flavonoids, terpenoids and phenolic compounds. The green synthesized NPs dose-dependent free radical scavenging activity and excellent antimicrobial activity. Further, the findings of the cytotoxic study against HCT-116 colon cancer cell line by MTT assay supports its broad-spectrum biological effects. All these results demonstrated that, the biocompounds present in the plant extract was useful for the synthesis of CuNPs with antibacterial and anticancer activity. Hence it is concluded that *U. indica* mediated green synthesized Cu-Cu_2O NPs may lead to beneficial discoveries in anticancer drug. Hence, these stabilized nanoparticles can be a potent drug having various biomedical applications.

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7. AUTHORS CONTRIBUTION STATEMENT

Dr. H.R.Raveesha conceptualized and prepared the original draft. The whole study and the preparation of the manuscript was designed including antimicrobial studies and antioxidant effects at the Departmentof Botany, Jnanabharathi, Bangalore University, Bengaluru. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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