



Anticancer Activity of Copper Oxide Nanoparticles Synthesized from *Brassia actinophylla* Flower Extract

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There are many methods to synthesize metal and metal oxide nanoparticles. In this paper, copper oxide nanoparticles have been synthesized by solution combustion method using *Brassia actinophylla* i.e. *Schefflera actinophylla* flower extract belongs to *Araliaceae* family. The importance of solution combustion is one of the easy and simplest methods for the synthesis of metal oxide nanoparticle. The CuO nanoparticles were synthesized at various temperatures and the characterization has been carried out by UV, FTIR, PXRD, SEM, TEM and EDAX analysis. At lower temperature, the peak was not observed but at 400 °C, the UV peak was observed at 340 nm. The FTIR peaks observed at 1000-500 cm⁻¹ confirms again the presence of CuO nanoparticles. The monoclinic phase and crystalline structure of nanoparticles were revealed by PXRD pattern, by Scherrer's method the average crystalline sizes were found to be in the range of 15 to 24 nm. The size and the shape of nanoparticles were confirmed by SEM and TEM reports. The SEM images of nanoparticles show spherical in shape and free from agglomeration. TEM analysis reports the nanoparticle sizes ranging from 2 to 20 nm. The percentage of copper (52 %) and oxygen (26 %) elements were recorded in the EDAX analysis. The study of size and stability of nanoparticles were done by zeta potential values. The antibacterial activity of CuO nanoparticles were carried out against *Staphylococcus aureus* and *Escherichia coli* bacteria's by agar well diffusion method. The MTT assay was performed in order to check the anticancer activity of CuO nanoparticles against HT-29 colon cancer cells.

Keywords: *Brassia actinophylla*, CuO nanoparticles, Anticancer activity, Zeta potential.

INTRODUCTION

There are different methods to prepare nanoparticles such as physical, chemical and biological methods [1-4]. It is difficult to achieve the controlled size and stability of nanoparticles by chemical reduction method, but the green synthesis of nanoparticles using plant extracts is friendly to nature and inexpensive [5-7]. The green synthesis of nanoparticles process involves various parts of the medicinal plants as reducing agents. The extracts prepared from leaves, stem, roots, flowers and the seeds of medicinal plants were employed as a reducing agent for the nanoparticle synthesis. Nanoparticles like copper, zinc, silver and their oxides (CuO, ZnO and AgO) were synthesized by using above method [8]. The nanoparticles synthesized by green synthesis method is stable. The reducing agent used in chemical method such as sodium borohydride and hydrazine

causes skin, eye, nose and throat irritation. The toxic chemicals released during chemical synthesis of nanoparticles causes difficulties to all the living things and environment. The more usage of above chemicals affects the nervous system [9]. The pulse laser ablation, microwave-assisted and pulsed/explosion wire discharge methods are physical methods to synthesize nanoparticles. The efficiency of the laser method is less but its high cost and high energy consumption method [10]. The radiations produced in microwave assisted synthesis are hazards to health. The pulsed/explosion wire discharge method has some contaminations in the final product [11]. Because of the above impulsion, medicinal plant extracts are put to use to synthesize nanoparticles. Now a days many nanoparticles were synthesized using plants such as neem [12], *Cinnamomum camphora* [13], lemon grass [14], tamarind [15] and *Euphorbia tirucalli* [16]. Metal oxides mainly CuO nanoparticles have

various applications like catalytic [17], electrical [18], optical [19] and superconductors [20]. The optical and electrical properties are highly beneficial to produce multipurpose nano-sized electronic and opto electronic devices [21-23]. The band gap of CuO nanoparticles is 1.2eV and these nanoparticles are p-type semiconductors, used in industries such as gas sensing, solar energy conversion, magnetic storage media, field emission devices and batteries [24]. CuO nanoparticles have variety of biological activities including antibacterial, anticancer, anti-diabetic, anti-inflammatory, antioxidant and wound healing [25-27].

Brassia actinophylla is also called as *Schefflera actinophylla* [28]. The leaves forming umbrella-like symmetrical heads so it is named as Umbrella tree. It grows up to 100 feet. The phenolic and flavonoid compounds are present in this plant shows anti-oxidant activity [29]. There are several routes to prepare CuO nanoparticles, namely microwave irradiations [30], thermal decomposition [31], sol-gel [32] and solid-state reaction method [33]. In a simple and easiest way, metal oxides can be prepared by solution combustion method [34]. Herein, CuO nanoparticles were prepared using *Brassia actinophylla* flower extract by solution combustion method. The synthesized CuO nanoparticles were mainly taken for the antibacterial activity against Gram positive and Gram negative bacteria's. The CuO nanoparticles were checked for the anticancer activity using HT-29 colon cancer cells.

EXPERIMENTAL

Cupric nitrate hexahydrate [$\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] 98 % pure was purchased from Fischer-Scientific, India. The plant was collected from New Horizon College Campus, Bangalore, India and authenticated by Institute of Trans-Disciplinary Health Sciences and Technology, Yelahanka, India. Scientific classification of plant is Plantae Kingdom, Apiales Order, Araliaceae Family, *Schefflera* genus, *S. actinophylla* species and binomial name is *Schefflera actinophylla*, Synonyms: *Brassia actinophylla*.

The UV-visible spectrum of synthesized CuO nanoparticles were measured by UV-2301 (Techcom) spectrometer. FTIR spectrum was recorded for evaluate the organic and inorganic constituents in wavelength ranging from 4000-500 cm^{-1} through Nicolet iS5 FTIR Spectrometer (Thermo Fisher). EDAX and PXRD analysis were carried out by X-ray diffractometers (PANalytical) instrument. Morphology study of green synthesized nanoparticles was carried out from SEM (TESCAN-VEGA3) analysis. Size and morphological analysis of nanoparticles were done by TEM (Jeol/JEM2100) instrument. Further, stability of green synthesized nanoparticles was determined by zeta potential analysis.

Preparation of plant extract: *Brassia actinophylla* flowers were collected and dried under the absence of sunlight condition, after that it was grained to powder form and taken for extraction process using Soxhelt apparatus around 70 h at 45-60 °C. The aqueous solution was subjected to concentrate by using rotary flash evaporator (Buchi, Flawil, Switzerland) at 40 ± 5 °C under reduced pressure. Then concentrated extract was dried in hot air oven at 50-60 °C, eventually the dried crude extract was utilized for the synthesis of nanoparticles.

Synthesis of CuO nanoparticles: To synthesize CuO nanoparticles, 0.21 g of dried crude flower extract of *B. actinophylla* and stoichiometric amount of cupric nitrate hexahydrate was dissolved in 10 mL of distilled water with constant stirring about 10-15 min to attain the homogeneous condition and the mixture solution was kept in a pre-heated in muffle furnace maintained at 100 ± 10 °C for 10-12 min. And the above condition was repeated and calcinated for various temperatures (200 °C and 400 °C). The black coloured powder materials were obtained and cooled to room temperature afterwards stored it in airtight container for further studies [35].

Antibacterial activity: The agar well diffusion method has been employed to notice the antibacterial activity against *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram -ve) for the synthesized CuO nanoparticles [36,37]. Then wells were prepared in the plates with a cork borer. Each well was loaded with various concentration of samples such as 300, 450, 600, 750, 900 $\mu\text{g}/\text{mL}$. For positive control, 10 mg of tetracycline dissolved in 1 mL of 10 % DMSO was used. The plates were incubated for 24 h by maintaining the temperature at 37 °C.

Anticancer activity: The log phase colon cancer cells (HT29) were suspended in the complete DMEM media to make a final cell concentration of $4 \times 10^3/\text{mL}$ cells. In a tissue-culture coated 96-well plate, 150 μg of these cells were seeded and incubated at 37 °C overnight supplemented with 5% CO_2 . After that, when cells were adhered to plate surface, the given samples was supplemented at different concentration to make the final volume of 200 μg . The concentrations used were 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$. All the concentrations were added as triplicates along with control *i.e.* media without cells and cells without test samples. After 24 h, 20 μg of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37 °C. Then, 100 μg of DMSO (to solubilize the purple formazon) was added, incubated for 0.5 h, after which the absorbance at 570 nm was recorded with a microplate reader (Thermoscientific Multiskan FC) [38,39]. Growth cancer cells were expressed as the percentage of cell viability.

RESULTS AND DISCUSSION

UV-visible analysis: The formation of green synthesized CuO nanoparticles were monitored by UV-visible spectrum at various temperatures such as 100, 200 and 400 °C (Fig. 1). Initially at 100 °C, there is no absorption peak appeared which indicates the absence of nanoparticle formation. Subsequently at 200 °C also did not exhibit any peaks in spectrum. Because decomposition of organic material was not completely carried out therefore no absorption peak arises for nanoparticles formation. While at 400 °C calcination, fine black colour power was obtained and subjected UV-visible spectrum has revealed a peak at 340 nm which confirmed the formation of CuO nanoparticles [40].

FT-IR analysis: Formation of the green synthesized CuO nanoparticles were confirmed by FT-IR analysis. FT-IR analysis of 100 °C calcinated material has revealed different vibration peaks like 2913 cm^{-1} , which is due to C-H stretching vibration, the band at 2359 cm^{-1} shows the O=C=O vibration, 1386 cm^{-1} for bending vibration of methyl bending and 1032 cm^{-1} indicates

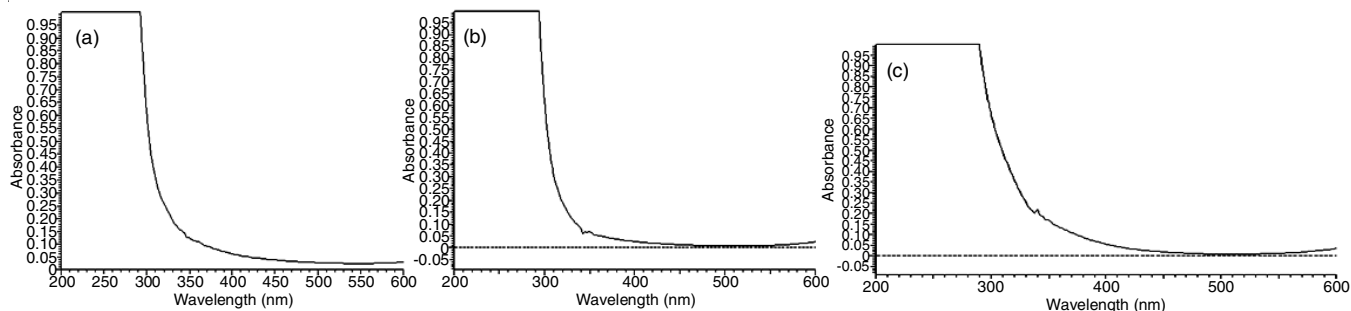


Fig. 1. (a-c) UV-visible spectrum of green synthesized CuO nanoparticles at various temperature of combustion from *Brassia actinophylla* flower extract

the -C-O stretching vibration. At 200 °C calcination, FT-IR analysis exhibited 3446 cm^{-1} peak for C-H stretching and 2917 cm^{-1} for aliphatic C-H stretching. A sharp peak at 2359 cm^{-1} shows the O=C=O bending vibration. The peaks at 1653, 1636, 1558 cm^{-1} represented the -C=O stretching and 1025 cm^{-1} peak observed for -C-O stretching. However, at 400 °C calcinated material of green synthesis has revealed the sharp peaks at 537 cm^{-1} in spectrum (Fig. 2), which indicates the metal oxygen (M-O) vibration [41]. And along with above observed peaks of organic compounds were also detected in 400 °C calcinated material, which portrayed the plant active compounds are involved in the nanoparticles synthesis. The FT-IR analysis exhibited the metal oxygen vibration peak (537 cm^{-1}) in 400 °C calcinated green synthesized material only. Hence the results of UV-Visible spectrum and FT-IR spectrum analysis confirmed the formation of CuO nanoparticles was achieved in 400 °C calcination.

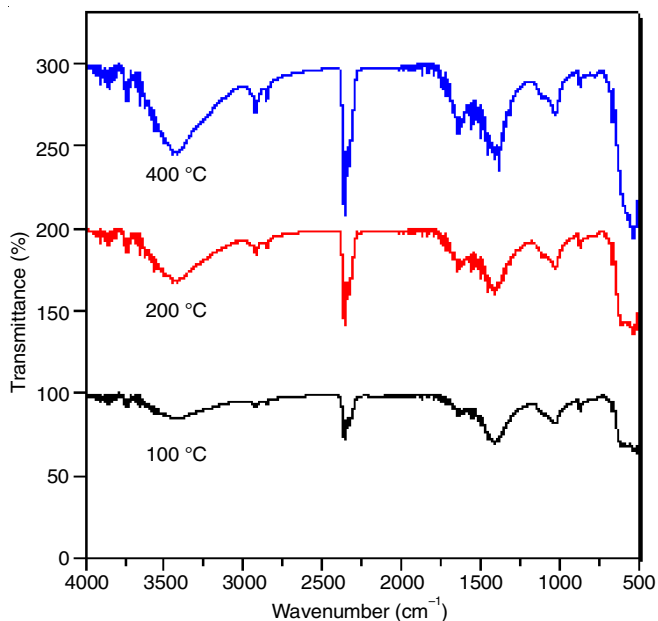


Fig. 2. FT-IR analysis of green synthesized CuO nanoparticles *Brassia actinophylla* flower extract

PXRD and EDAX analysis: The synthesized CuO nanoparticles were subjected to explore its crystalline properties by powder XRD analysis (Fig. 3). The peaks were observed in phase like 002 (111), 111(200), (202, 020, 202, 113). The broadened peak indicates the size of the particles were small and monoclinic. However, the XRD peaks are well matched

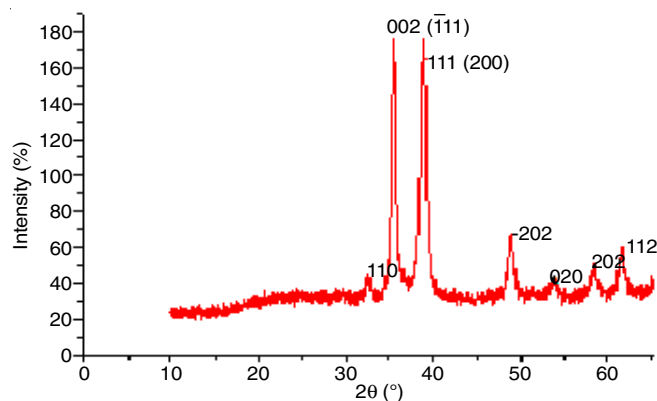


Fig. 3. Powder XRD analysis of green synthesized CuO nanoparticles *Brassia actinophylla* flower extract

with standard of Joint Committee on Powder Diffraction data (JCPD Card no: 05-0661). The less number of impurity peaks of CuO nanoparticles were observed in XRD pattern indicates the high phase purity.

Debye-Scherrer's formula $D = k\lambda/\beta\cos\theta$ used to calculate the average crystalline size [42]; where k is equal to 0.94, λ is the wavelength of X-ray radiation (1.5406 Å), β is the full-width at half maximum (FWHM) and 2θ is the Bragg angle (in degree). The average crystalline size of green synthesized CuO nanoparticles found to be in the range of 15 to 24 nm. The diffraction peaks were recorded at 35.6°, 38.9°, 49°, 61.8°, 66.27° angles which corresponds to the lattice (002) (111), (111) (200), (202) (020), (113).

The EDAX analysis exhibited the presence of elements composition in green synthesized CuO nanoparticles using aqueous extract of *Brassia actinophylla* flower. In synthesized nanoparticles, copper element was recorded as 52 % and oxygen recorded as 26 %. Besides other elements such as carbon (19 %) and potassium (2 %) was recorded as insignificantly (Fig. 4).

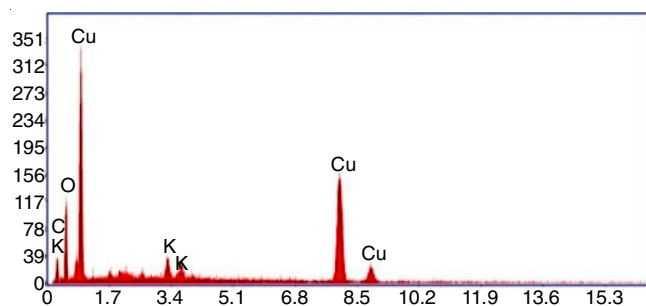


Fig. 4. EDAX analysis of green synthesized CuO nanoparticles from *Brassia actinophylla* flower extract

SEM analysis: The morphology of synthesized CuO nanoparticles were investigated by SEM analysis. The SEM image (Fig. 5a) at 10 μm magnification shows the nanoparticles as porous material, due to liberation of organic and gaseous molecules. In higher magnification at 500 nm has revealed the synthesized CuO nanoparticles are spherical in shape and having less agglomeration (Fig. 5b). However, more or less same morphological observation was noted in earlier report for synthesis of CuO nanoparticles using aqueous extract of *Gloriosa superba* L. leave [43].

TEM analysis: The exact size and morphological appearance of *Brassia actinophylla* flower aqueous extract mediated green synthesized CuO nanoparticles were examined by TEM analysis. The synthesized CuO nanoparticles shown in Fig. 6a are spherical in shape and the particles having less aggregation. The size of CuO nanoparticles were observed in range of 2-20 nm. Characteristics of nanoparticles varied by its size. If nanoparticles having very small size then it will have higher surface area and high reactive sites, thus nanoparticles has been studied widely because they possess more interaction and permeability within the cell wall metabolites of living things [44]. Hence, the size of synthesized nanoparticles has much concentration

to furnish effectiveness biological applications of it. The SEAD pattern of synthesized CuO nanoparticles (Fig. 6b) confirms the nanoparticles are in crystallite nature.

Zeta potential: Zeta potential analysis has been widely used to examine the stability of nanoparticles in a dispersed medium. Nanoparticles with a zeta potential between -10 to +10 mV are considered approximately neutral and increases considered as moderately stable. If zeta potentials values of the nanoparticles are greater than +30 mV or less than -30 mV are considered as highly stable [45]. In this process, synthesized CuO nanoparticles zeta potential were detected as -13.2 mV (Fig. 7). The zeta potential value of *Brassia actinophylla* flower aqueous mediated synthesized CuO nanoparticles ensure the nanoparticles possess moderate stability.

Anticancer activity: The biological application of *Brassia actinophylla* aqueous flower extract mediated synthesized CuO nanoparticles was evaluated by *in vitro* anticancer activity against colon cancer HT-29 cell line. The cancer cell living time and its proliferation have been examined by MTT assay based on the reduction of tetrazolium salt. In the present study, the synthesized CuO nanoparticles were taken in various concentration like 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$ (Fig. 8). The percen-

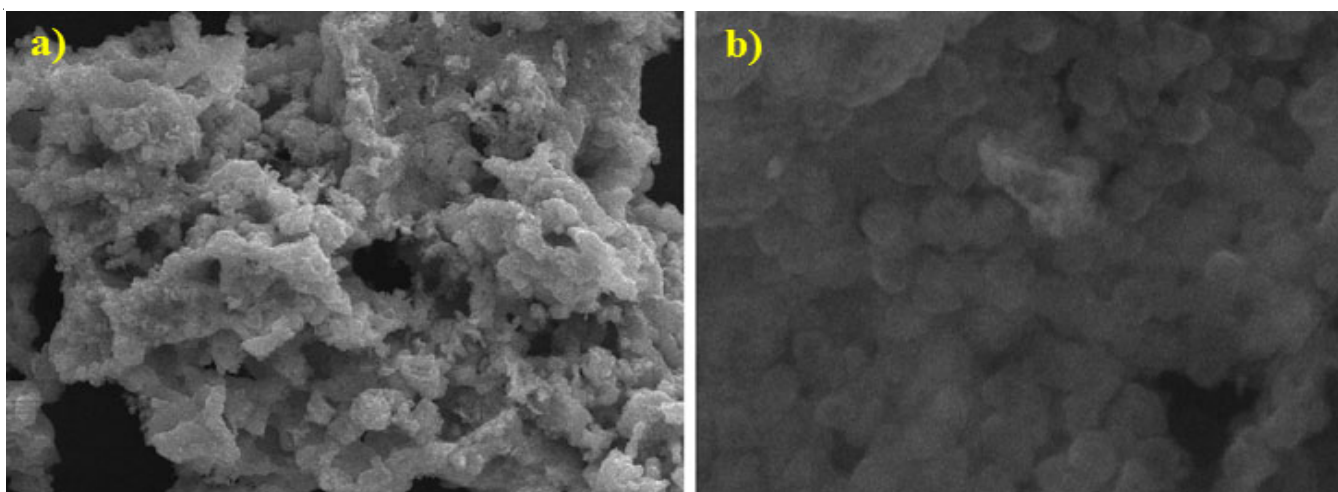


Fig. 5. (a,b) SEM images of green synthesized CuO nanoparticles from *Brassia actinophylla* flower extract

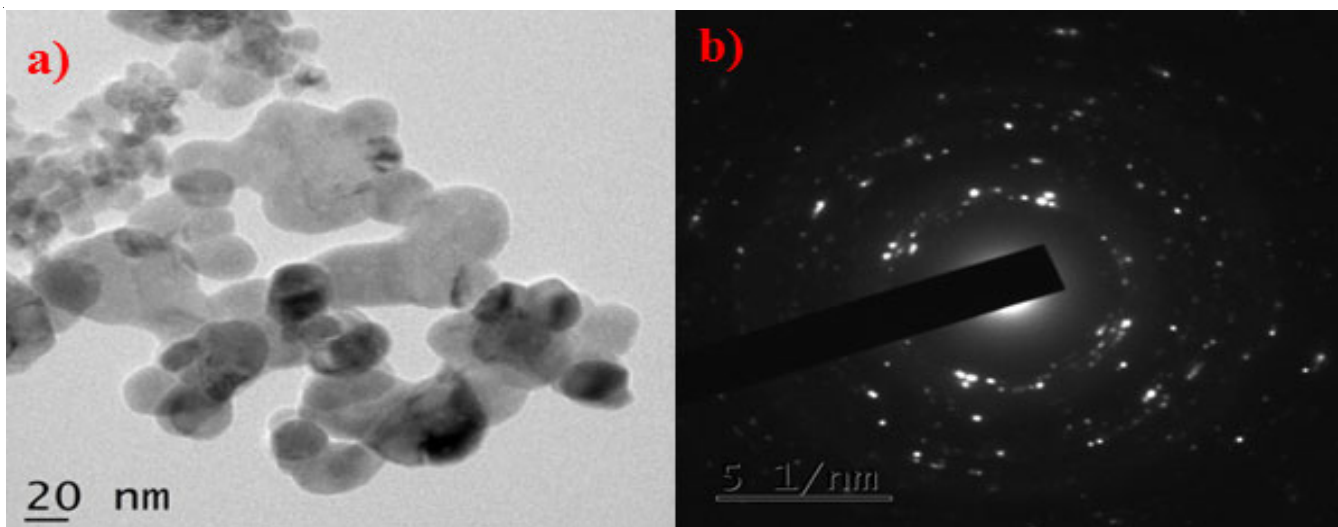


Fig. 6. (a,b) TEM analysis of CuO nanoparticles from *Brassia actinophylla* flower extract

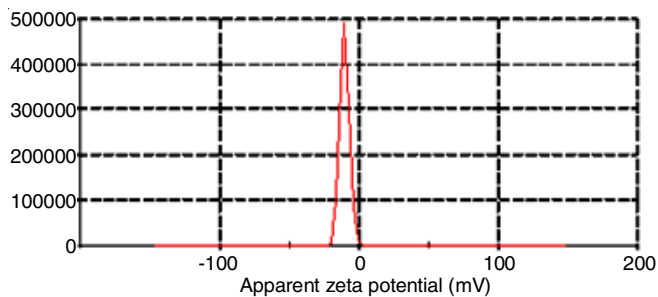


Fig. 7. Zeta potential analysis of CuO nanoparticles synthesized from *Brassia actinophylla* flower extract

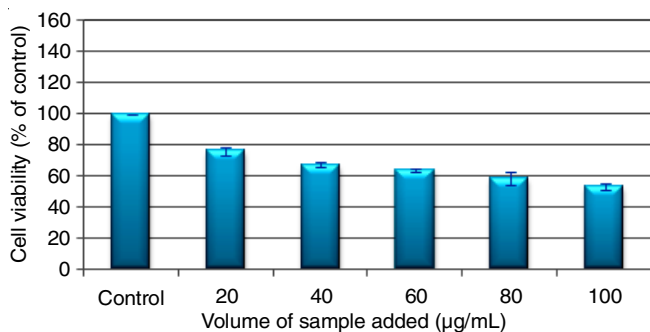


Fig. 8. MTT assay graph for anticancer activity of green synthesized CuO nanoparticles from *Brassia actinophylla* flower extract

tage of cell viability was calculated from OD value of MTT assay (Table-1). The synthesized CuO nanoparticles have revealed poor cytotoxic effect on the colon cancer cell line at initial concentration (20 µg/mL) shows 80 % cell viability. Subsequently increasing the concentration of green synthesized CuO nanoparticles the viability of cancer cell decreased. At higher concentration of CuO nanoparticles (100 µg/mL) exhibits 53 % of cell viability, which proved the synthesized nanoparticles consist potent anticancer activity against colon cancer.

Concentration of CuO NPs (µg/mL)	Cell viability (%)	Concentration of CuO NPs (µg/mL)	Cell viability (%)
0	100	60	64
20	80	80	58
40	67	100	53

While synthesizing the metal nanoparticles using plant extract which enhance their biological activity because biological effective compounds of plant are significantly load on its surface. Anticancer efficiency of green synthesized CuO nanoparticles has been studied for breast cancer (MCF-7), cervical cancer (HeLa), epithelioma (Hep-2) and lung cancer (A549) [46]. The anticancer activity of *Brassia actinophylla* flower aqueous extract mediated green synthesized CuO nanoparticles (100 µg/mL) has moderate activity against to colon cancer cell proliferation.

Antibacterial activity: Antibacterial activity of *Brassia actinophylla* flower extract mediated green synthesized CuO nanoparticles also examined to evaluate its antimicrobiological application. The green synthesized CuO nanoparticles were explored against human pathogenic bacteria's such as *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram

-ve). The least antibacterial activity was observed at lower concentration exhibits zone of inhibition about 12 mm. Moreover, increasing the concentration of CuO nanoparticles has revealed higher activity against both gram positive bacteria and gram negative bacteria and simultaneously the highest zone of inhibition was observed by the control drug tetracycline for both organisms (33 and 30 mm). Although green synthesized CuO nanoparticles exposed the higher zone of inhibition at 900 µg/mL concentration for both organisms. Table-2 shows the green synthesized CuO nanoparticles has potent antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*. *Brassia actinophylla* flower has abundance of carboxyl groups [47] on cell surface and high affinity of copper toward these carboxyl groups [48]. Thus CuO nanoparticles have revealed potent antibacterial activity against gram positive bacteria than gram negative bacteria. Spherical shape of nanoparticles and size in the range 2 to 20 nm could increase the biological activity.

Microorganism	Conc. (µg/mL)	Zone of inhibition (mm)	
		Sample	Control*
<i>Staphylococcus aureus</i>	300	12	33
	450	14	
	600	17	
	750	17	
	900	19	
<i>Escherichia coli</i>	300	12	30
	450	14	
	600	15	
	750	15	
	900	16	

*Control: Tetracycline (300 µg/mL)

From the above results, it is clear that the antibacterial activity of both Gram +ve and Gram -ve bacteria's are above 9 mm (zone of inhibition) so the antibacterial activity of CuO nanoparticles are good in *Brassia actinophylla* flower extract compared to *Rauwolfia serpentine* [35]. The cell viability of CuO nanoparticles at the concentration of 20 µg/mL observed was 80 %. It shows that at lesser concentration, anticancer activity of CuO nanoparticles synthesized from *Brassia actinophylla* flower are high [46].

Conclusion

The synthesis of CuO nanoparticles using *Brassia actinophylla* flower extract has been carried out successfully by solution combustion method. The combustion was done at three different temperatures and CuO nanoparticles were achieved at 400 °C. The biosynthesized CuO nanoparticles were confirmed by UV-visible and FT-IR spectrum studies, which clearly indicates the biomolecules present in *Brassia actinophylla* flower potentially reduce the metal ions into metal nanoparticles and also act as green stabilizing agent. Powder XRD pattern confirmed the monoclinic crystalline nature of synthesized CuO nanoparticles. The morphological analysis of CuO nanoparticles ascertained through SEM images, displayed the particles are free agglomerated with spherical in shape. TEM images of CuO nanoparticles revealed that size of nanoparticle

is in the range of 2 to 20 nm. Zeta potential analysis ensures that the synthesized CuO nanoparticles by green method are moderately stable. The significant anticancer activity of nanoparticles for colon cancer and antibacterial activity against selected pathogenic bacteria are supports to establish the biological and pharmaceutical applications of green synthesized CuO nanoparticles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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