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ORIGINAL ARTICLE **OPEN ACCESS**

Therapeutic potential of bioactive copper oxide nanoparticles (CNPs) towards breast cancer, lungs cancer and prostate cancer

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ABSTRACT
Copper oxide nanoparticles (CNPs) were created from copper sulfate solutions (2M) using plant extracts from Abies pindrow royle and Leucas cephalotes. CNPs were evaluated using UV-visible, XRD, FTIR and scanning electron microscopy coupled with energy dispersive X-ray and zeta potential. UV-vis spectrophotometer examination was utilized to monitor the growth and stability of reduced CNPs in the colloidal solution. XRD analysis reveals that the crystalline average size of the synthesized CNPs was 33.60 nm and 23.1 nm. SEM analysis reveals that synthesized were spherical in shape with their average size less between 10-30 nm. The antitumor activity of the biogenic synthesized copper oxide nanoparticles against PC-3 (prostate cancer cell line), Hela, A459 (human lung cancer cell line), and MCF-7 (human breast cancer cell line) was evaluated using MTS and MTT tests. CNPs produced from A. pindrow royle leaf extracts demonstrated a substantial anticancer impact against MCF-7 breast cancer (IC50 = 1.09 M), according to the observed IC50 value.
Keywords: *green synthesis CNPs, characterization, anticancer activity.*

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INTRODUCTION

Metal oxide nanoparticles are generally helpful in chemical science, material science and climate science due to uniqueness in their physical and chemical properties. Due of their unique high surface area to volume ratio and high density, these manufactured nanoproducts generates wonderful result and several applications in a variety of areas such as nanotechnology, medicine, biology, agriculture, sensors, solar cell, catalysis, photo degradation of dyes, electronic etc. [1-4]. The nanoscale size of metallic nanoparticles permits several connections with bioactive molecules at the outer surfaces of cells and inside the cells in such a manner which may be decoded and responsible for the several biochemical and physio-chemical characteristics of these cells. Furthermore, their probable utilization in drug-delivery system and non-invasive imaging gives several advantages against traditional and conventional biomedicinal agents [5]. There are various types of metallic oxide nanoparticles such as ZnONPs, CNPs, TiO_2 NPs etc. Now a days, CNPs were amongst the preferred researched noble metals among several scientists and researcher, because of their specific and immense photo degradation, biological and photocatalytic activity. [6]and resemblance with other metal oxide and metallic NPs. [7]CNPs is a semi - conductive substance having several properties such as electrical, piezoelectrical, optical and magnetic [8] and its formulation has incited huge attention among scientist because of its extensive variety of applications.
Biological techniques for synthesizing NPs using plant leaf extracts or organisms like fungi, bacteria, and algae are considered eco-friendly substitutes for chemical synthesis because such approaches are nontoxic and less cost- and energy-intensive [1]. Plant extract-based NP production approaches are more beneficial than environmentally friendly biological approaches because cell cultures are not required [2]. Moreover,

NP production using plants is beneficial because of safe handling and easy availability associated with plants and their extensive metabolite content to facilitating reduction [3].
Moreover, phytochemicals like flavones, tannins, aldehydes, resins, polyphenolic acid, alkaloids etc., were observable in the extracts of diverse plant parts (fruits, peel, bark and seeds) acting as a capping, reducing and stabilizing agent of CNPs. [9]Moreover, plant-mediated CNPs have been largely utilized in a wide range of chemical and biological application, including cytotoxicity antimicrobial activity[10] anticancer activity [11-15] photodegradation and photocatalytic activity [16-18]. Since CNPs are crucial in the field of pharmaceutical drugs because of their potential antibacterial and anticancer activities, therefore we attempted the green fabrication of CNPs produced from *Abies pindrow* royle and *Leucas cephalotes* leaf extract and then we evaluated their antimicrobial activities and anticancer activities.

MATERIAL AND METHODS

Fresh and healthy leaves of *Abiespindrow* royle and *Leucas cephalotes* were collected from Kotdwara, Pauri Garhwal, Uttarakhand and authenticated from National Tropical Research Institute of Forest, Jabalpur, Madhya Pradesh, Species number is UK2252 and UK3745.Copper sulphate ($CuSO_4$) and sodium hydroxide (NaOH) was provided by Department of Chemistry, Motherhood University, Roorkee Uttarakhand. The double distilled deionized water was used in all the experiments.

Preparation of *Abies pindrow* royle and *Leucas cephalotes* leaf extract:

Fresh and healthy leaves of both plants *Abies pindrow* royle and *Leucas cephalotes* were thoroughly washed with deionized water in order to take out any of the contaminants and sticky dirt. After 15 days of tint drying, the leaves of both plants must have reached their desired constant mass. Then, stale leaves of both the plants were mashed with mortar-pestle and then the Using 7 g of finely powdered leaves from each plant and 150 ml of double-distilled water in a 250 ml Erlenmeyer volumetric flask, a 15-minute heating process at 65–70 °C was used to create the leaf extracts from both plants. The extracts from both plants were then filtered using Whatman filter paper no. 1 after becoming allowed to cool to room temp.

Synthesis of CNPs

From leaves extract of *Abies Pindrow Royle*

An aqueous solution of 5 mM CuSO4 was produced in order to produce copper oxide nanoparticles. Then, 50 ml of a 5 mM CuSO4 aqueous solution and 9 ML of *Abies Pindrow* Royle leaf extract were integrated for the bio reduction procedure. Then, as shown in figure 1, 0.1 M NaOH was gradually introduced into the reaction mixture to adjust the pH. Then it was heated for two hours, stirring continuously, to an internal temperature of 80 °C. After two hours, the solution mixture's color changed from yellow to a dark green**.** After that, the mixture was then allowed to spontaneously cool to room temperature. The pellets were isolated by centrifuging at 1500 rpm for 20 minutes and the isolated product was stored for further studies.

From leaves extract of *Leucas Cephalotes*

To create copper oxide nanoparticles, 25 mg of CuSO4 was dissolved into water. Then, 100 ml of flask were filled with 9 ML of *Leucas cephalotes* leaf extract. And after that, while being continuously stirred, it was processed to 80 °C. After 1 hour and 18 minutes, the color of the solution mixture changed from brown to green. Once at room temperature, the mixture was allowed to cool naturally. The contaminants were then washed away with deionized water after the pellets were separated by centrifuging at 1500 rpm for 15 minutes. In preparation for more research, the isolated product was stored.

In vitro cytotoxicity

Cell line culture

PC-3 (Prostate Cancer Cell Line), Hela (Human Endometrial Cancer Cell Line), A459 (Human Lung Cancer Cell Line), and MCF-7 (Human Breast tumor) cells were used in this assay.

MTS Assay against A459, Prostate cancer Cell Line

Method: MTS is a less toxic alternative to MTT assay, and the formazan developed from MTS is water soluble. The MTS Assay is often used to evaluate cell proliferation, viability, and cytotoxicity. The MTS assay method is based on the inhibition of viable mammalian cells with MTS tetrazolium compound to produce a colored formazan dye that is soluble in cell culture medium. The cell growth inhibition assay was performed according to the manufacturer's instructions by using cell titer 96 aqueous non-radioactive cell proliferation assay (Promega, WI, USA). MTS compound and the electron coupling reagent phenazine ethosulphate are both present in one solution (PES). Cells bio reduce the MTS compound (3-(4,5-dimethylthiazol-2-yl)-5-(-3-carboxymethoxyphenyl)-2(4-sulphonyl)-2H-tetrazolium) to a colored formazan product that is soluble in tissue culture medium. A459 (Human Lung Cancer) Cell Line was transferred 24 hours before treatment to 96-well tissue culture plates at a density of 5000 cells/well. The medium was then replaced with a new medium containing PTAE or copper oxide nanoparticles at different doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 μg mL). The control group received culture medium that was

devoid of any drug formulation. Following 72 hours of incubation at 37°C and 5% CO_2, the media was removed, and the rinsed thoroughly twice with sterile PBS. Each well received 20 l of MTS solution (0.5 µg/ml) and was incubated at 37 digree for 4 hours. Following the removal of the medium, each well received 100 ml of DMSO to solubilize the formazan crystal created from MTS. Cell viability was ascertained employing spectra at 490 nm and a citation at 690 nm with a Max 340 microplate reader (Molecular Devices, USA).

MTT Assay for MCF-7 and HeLa cells

The MTT colorimetric assay (dimethylthiazol-diphenyltetrazolium bromide) helps determine mitochondrial viability and thus cell viability. A mitochondrial dehydrogenase enzyme in living cells gets converted yellow tetrazolium MTT salt to blue MTT formazan, which results in undamaged cells.

Method: MTT assay was conducted the cytotoxic nature of MNPs in MCF-7 (human breast cancer) cell lines. In 96-well plate, 5,000 MCF-7 cells were seeded and incubated for a 24-hour period. Cells were also treated with TiO2NPs at various doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg) and 72 hours incubation. After that, the cells were exposed for four hours to ten litres of freshly prepared yellow MTT reagents (0.5 µg/mL). Finally, 100 µL of dimethyl sulfoxide (DMSO) was introduced, and the violet formazan solution's UV absorbance at 570 nm was measured (Multimode reader, Tecan, Austria).

Characterization:

Using an Elite-double beam UV-visible spectrophotometer, the first step in examining the plant-mediated synthesis of CNPs was done. CNPs were then subjected to XRD analysis using a P analytical X'PERT-PRO Diffractometer (CuK radiation, max = 1.54), and their XRD spectra were found to be in the range of 2 from 0° to 80°. In order to pinpoint the bioactive plant extract molecules that were in charge of capping and stabilizing the CNPs, Fourier transform infrared spectroscopy (Spectrophotometer Perkin Elmer Model RZX) analysis was used. To determine the dimensions and shape of CNPs, scanning electron microscopy has been used. It was possible to use EDX to recognize the elements that make up synthesized nanoparticles.

RESULTS AND DISCUSSIONS

Using aqueous leaf extract from *Abies pindrow* royle and *Leucas cephalotes*, the new research successfully achieved plant-mediated synthesis of CNPs. A number of phytoconstituents found in both plant's aqueous extracts were crucial in the manufacturing of CNPs by plants. Surface plasmon resonance (SPR) influenced the solution turned to modify from yellow to bright green in the case of *Abies pindrow* and from brown to green in the case of *Leucas cephalotes*. This colouration may have been triggered by the formation of of CNPs.

Spectra

UV-Visible

Figure1 depicts the UV-visible spectrum of the *Abies pindrow* and *Leucas cephalotes* solutions having a broad absorption peak at λ_{max} = 241 nm and λ_{max} =347 and 372 nm respectively which validated the presence of CuO nanoparticles in their respective solutions and their calculated energy band gap was found to be 5.15 eV and between 3.33- 3.57 eV in case of *Abies pindrow* and *Leucas cephalotes* respectively.

XRD

copper oxide nanoparticles identified the (020), (113), and (221) diffraction planes of monoclinic copper oxide (shown in figure2a). These findings correspond to copper oxide nanoparticles found in *Cyperus rotundus* leaf extract [19]. The XRD patterns utilizing *Leucas cephalotes* extract are (displayed in Figure2b). The miller indices values were (110), (002), (111), and (202), respectively, and the diffraction peaks were found at 32.44, 35.37, 39.86, and 47.06. These diffraction peaks show that both CuO is naturally crystalline. The spectrum is similar to that of pure CuO, which suggests that the monoclinic single phase CuO that was formed in the current experiment. The width of the peak and the size of the nanoparticles are inversely correlated, meaning that as the peak width widens, the size of the nanoparticles narrows, indicating the existence of material in nanorange. Using the Debye-Scherrer formula the average crystalline size of the copper oxide nanoparticles synthesized from *Abies pindrow* and *Leucas cephalotes* are 33.60 and 23.01 nm respectively. These findings correspond to copper oxide nanoparticles were comparable to the literature work. [20, 7]

FTIR

FT-IR spectra of CNPs displayed various peaks which validate the presence of various functional groups of phytochemicals that were responsible for the capping and stabilizing of nanoparticles. FT-IR peaks of CNPs using *Abies pindrow* leaf extract in Figure 10, at 2885 cm^{-1} corresponds to stretching vibrations of carboxylic (COOH) group. Further, peaks at 1610.8 cm^{-1}, 1519 cm^{-1}, and 1337.9 cm^{-1} may be attributed to C=O frequency of extensively conjugated systems, C=C aromatic stretching and C-O stretching of ArOH respectively. Peaks below 600 cm^{-1} regions may be attributed to the CNPs. FT-IR peaks of CNPs using *Leucas*

cephalotes leaf extract in Figure 11, at 3738.05 cm^{-1} corresponds to stretching vibrations of hydroxyl (OH) group. Further, peaks at 1697.36 cm^{-1}, 1514.12 cm^{-1}, and 1217.08 cm^{-1} may be attributed to C=O frequency of extensively conjugated systems, C=C aromatic stretching and C-O stretching of ArOH respectively. Peaks below 600 cm^{-1} regions may correspond to the CNPs. Thus, the FT-IR spectra reveal the presence of bioactive components present in the leaf extract of *Abies pindrow* royle and *Leucas cephalotes* that were responsible for the synthesis and stability of synthesized CNPs These FTIR spectral results were comparable to the previous findings as reported in various literatures [21]

Morphology

SEM analysis was used to study the surface morphology of CNPs using *Abies pindrow* royle (Figure 3a) and *Leucas cephalotes* (Figure 3b). These results confirmed that plant mediated synthesized CNPs of both the plants were spherical in shape with their average size less between 10-30 nm. These SEM results were also comparable to the literature work. [20]. EDX was carried out for the elemental composition of CNPs using *Abies pindrow* royle and *Leucas cephalotes*. Sharp signal which confirms the presence of elemental CuO(Cu-58% , O-38.04% and Cu-53.21%, O-24.14%) in both cases using *Abies pindrow* royle and *Leucas cephalotes* respectively. The other signals that were noticed in the spectra may be attributed due to the bioactive components present in plant extract. These findings were also observed in several literatures. [21, 22]

CNPs Cytotoxicity study against PC-3 (Prostate Cancer) cell line, HeLa cancer cells line

CNPs inhibited PC-3 tumour growth in a concentration-dependent manner. Its IC50 values were 1.65 and 1.69 µM, respectively. CNPs have previously been shown to exhibit anticancer effect against prostate cancer cells. Recent research has demonstrated that CNPs can suppress angiogenesis, a mechanism that is essential for cancer cell development and dissemination. CNPs inhibited the growth of HeLa cancer in a concentration-dependent manner. CNPs have previously been discovered to exhibit anti-cancer effects against a many of cancer cells [23]. The inhibitory concentration (IC50) of CNPs on HeLa cancer cells was found. Its relative IC50 values were 2.11 and 3.45µM. CNPs created using A.*pindrow* royle leaf extract had the lowest IC50 at 2.11 µM.

Cytotoxicity study of CNPs against A459 (Human Lung Cancer) and MCF-7 cancer cells line

CNPs suppressed the proliferation of A459 (Human Lung Cancer) cancer cells in a concentration-dependent manner. Synthetic CNPs were tested in vitro against A459 (Human Lung Cancer) at a variety of doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg/ml). The cytotoxic effect of the synthesized CNPs was greater at higher concentrations (11 to 100 g/ml) but decreased at lower concentrations (0.41 to 10 µg/ml). In A459 cells, copper oxide nanoparticles showed substantial anticancer activity and inhibitory concentration levels (Human Lung Cancer). IC50 values for copper oxide nanoparticles were 1.23 and 1.55µM, respectively. The cytotoxic experiment revealed that CNPs have significant anticancer action against MCF-7 cells at different doses.[24] Cell death in MCF-7 cells was considerably increased when the concentration of CNPs dose. In this work, *A. pindow royle and L. cephalotes* extracts generated the synthesis of extremely excellent CNPs with increased activity against cancer cells with IC50 values in the micro molar range of 1.09 and 1.75, µM, respectively.

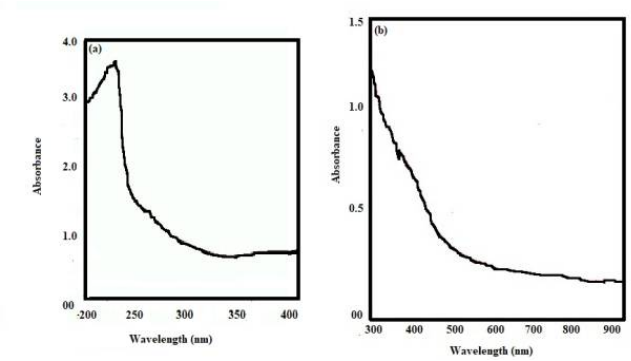

Fig. 1. U.V. visible spectra of CNPs (a) using *Abies pindrow* royle leaf extract. (b) using *Leucas cephalotes* leaf extract.

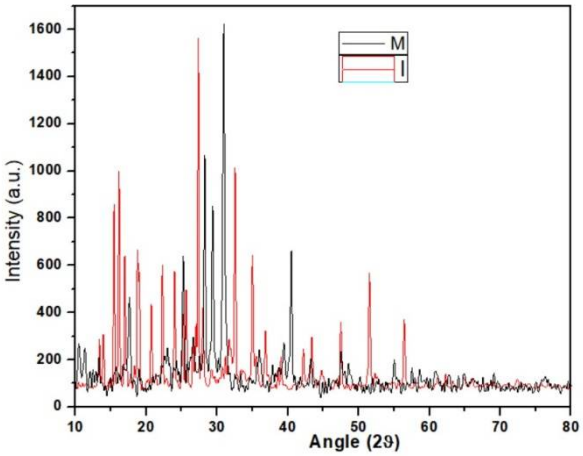

Fig. 2. XRD spectrum of CNPs (a) I- with *Abides pindrow royle* leaf extract (b) M- with *Leucas cephalotes* leaf extract.

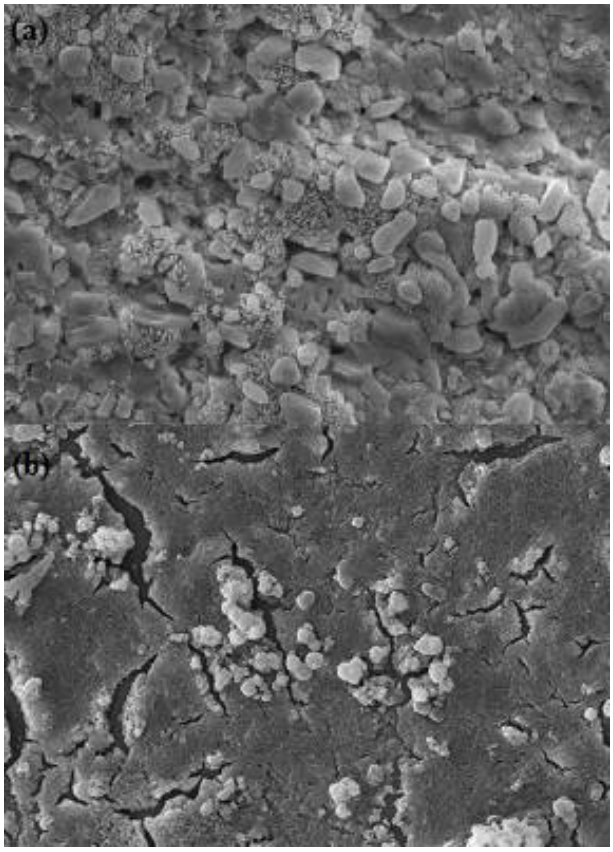

Fig. 3.SEM image of CNPs (a) with *Abies pindrow royle* (b) with *Leucas cephalotes*.

CONCLUSION

The green synthesis of CNPs from plants offers substantial opportunities in medicine and other sectors because of their biological applications and synthesis methods. Using the plant leaf extracts from *Abies pindrow* royle and *Leucas cephalotes* to make copper oxide nanoparticles. The green method of synthesis, which was noticed to be advantageous for the climate, can generate nanoparticles with less chemical input than the conventional techniques. Synthesized CNPs had an average size between 10 and 30 nm with spherical shape. EDX analyses were used to determine the structure's purity and composition. While the stretching and bonding were studied using FT-IR spectroscopy. This study also discovered that CNPs showed a stronger cytotoxic effect against MCF-7 (breast cancer) and A549 lungs in comparison to Hela and A549 lungs.

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