

Green Synthesis Of Copper Oxide Nanoparticles Using Lawsonia Inermis L. Root Characterization And Anti Cancer Potential

Nirubama Kumar^{1*}, Rekha Raja², Erraf Allabaji³

^{1*}Department of Biochemistry, Assistant Professor, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu – 641029, India

²Department of Botany, Assistant Professor, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu - 641029, India

³Department of Biochemistry PG Research, Research Scholar, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu - 641029, India

¹nirubamak@kongunaducollege.ac.in, ²rekka_bo@kongunaducollege.ac.in, ³errafmax007@gmail.com

***Corresponding Author**

Dr.K.Nirubama

Assistant Professor

Department of Biochemistry

Kongunadu Arts and Science College (Autonomous)

Coimbatore

Mial id: nirubamak@kongunaducollege.ac.in

Abstract

Medicinal plants have important place not only maintain the health and life of human beings and animals. India is the chief producer of medicinal plants and is rightly called “Botanical Garden of the World”. The plant *Lawsonia inermis* L. (family- Lythraceae) commonly known as Henna or Mehndi is known for its cosmetic properties. A preliminary qualitative phytochemical screening and anti-cancer properties of *Lawsonia inermis* root extracted with hydroalcohol, aqueous, Ethanol, Chloroform and Petroleum ether was investigated. The bioactive compounds are rich in hydroalcohol extract. Plant-mediated synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In the present study, we synthesized Copper oxide nanoparticles using hydro alcohol extract of root of *Lawsonia inermis* L. This method allowed the synthesis of nanoparticles, which was confirmed by ultraviolet-visible (UV-Vis) spectrophotometry and FTIR. UV-Vis spectra and visual observation showed that the colour of the root extracts of *Lawsonia inermis* L. turned into blue colour to greenish brown colour. The *in-vitro* anti-cancer activity in HT-29 colon cell lines was evaluated by MTT assay. The cell viability decreases while the cytotoxicity increases along with the increase in the concentration of the sample which indicates that it has potential cytotoxic effect against the colon cancer cell lines (HT-29). This plant has various medicinal properties such as antibacterial, antiviral, antimyotic, antimicrobial, anti-cancer etc. This plant is developing a innovative chemotherapeutic of pharma Industries for drug target identification.

KEYWORDS: Medicinal Plants, *Lawsonia inermis*, Phytochemical screening,

Copper Nanoparticles, Anti-cancer

1. INTRODUCTION

Medicinal plants are considered as vital resources of nutritive ingredients that can be used in drug development for their therapeutic values. Various plant species provide a rich source of bioactive compounds that are used to treat and inhibit some human disorders all over the world Nanotechnology is rapidly growing field with its application in science and technology for the purpose of manufacturing novel materials at the nanoscale level ^[1]. Further, the need for biosynthesis of nanoparticles developed as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemicals on the surface that may have adverse effect in the medical applications. ^[2]

Copper (Cu) is one of the indispensable microelements obligatory for the growth and development of plant. It can be present as Cu²⁺ and Cu⁺ under natural conditions. Optimum concentration is regularly involved in the plants, ranging from 10–14 to 10–16 M. In addition to many of its important functions such as cell wall metabolism and protein regulation, it also acts as secondary signalling molecule in plant cells. It takes part in the mitochondrial respiration, photosynthetic, electron transport, iron mobilization, hormone signalling, oxidative stress response, and also acts as cofactor for many enzymes.

Recently, there has been a steady increase in scientific reports on illness and death due to cancer, which requires effective treatments for its inhibition and control. The traditional approaches employed for the diagnosis and treatment of cancer cells have failed due to their inability to target the root cause of its lethal effects on normal cells and curb the severe side effects. To overcome this, several researchers and scientists are working to exploit various forms of nanostructured novel drug materials for targeting a wide range of cancer cells. These nanomaterials are the active candidate that can directly deliver medications to a precise site of the targeted cancer cells with reduced side effects. ^[3]

HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology. These cells are sensitive to the chemotherapeutic drugs 5-fluorouracil and oxaliplatin, which are standard treatment options for colorectal cancer. HT-29 cells have been well characterized over the years and are valuable to researchers that routinely use them as *in vitro* and *in vivo* models.

Lawsonia inermis L. is a perennial plant commonly known as Henna, belongs to lythraceae. Henna is cultivated by many farmers for cosmetic and pharmaceutical purposes, it belongs to the group of plants that are popular in nature and all parts of the plant (root, stem, leaf, flower, fruit and seed) are of great medicinal sources. The pharmacological studies showed that *Lawsonia inermis* showed antibacterial, antifungal, anti-parasitic, anticancer, antioxidant, hepatoprotective, anti-inflammatory, antipyretic, wound and burn healing, immunomodulatory, antidiabetic, antiulcer, antidiarrhoeal, anticancer and many other pharmacological effects. ^[4] In this context, here, an attempt has been made to synthesize copper oxide nanoparticles in an eco-friendly, greener route by using the important fern *Lawsonia inermis* L. ^[5] the potentiality of this CuONPs in the induction of defence and generation of stress has also been checked in a model plant *Lawsonia inermis* L. ^[6] Therefore, this study aims to explore the biomimetic synthesis and its efficacy as a source of nanomedicine against apoptosis and to establish their therapeutic values in anti-cancer potential of plant with nanotechnology has been highlighted. In future, these alternatives may be very useful in treating the chemotherapeutic agent in the pharmaceutical industry.

2. MATERIALS AND METHODS

Collection of plant sample

The root of *Lawsonia inermis* were collected from Erode situated between 11°16'32.30"N latitude and 77°35'16.58"E longitude. The root collected were labeled properly and brought to the laboratory. The samples were cleaned properly in tap water and shade dried at room temperature. The processed root were ground into fine powder and stored for further analysis.

Qualitative analysis of root extract of *Lawsonia inermis*

Test for Alkaloids

Wagner's Test: 0.5 ml of the bark and Heartwood extract was treated with Wagner's test reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and formation of reddish brown color indicates the presence of alkaloids. ^[7]

Test for Flavanoids

Sulphuric acid (H₂SO₄) test: A fraction of extract was treated with concentrated sulphuric acid and the formation of orange colour indicates the presence of flavonoids. ^[8]

Test for Tannins

Braymer's test: 0.5ml of extract was treated with 10% alcoholic ferric chloride solution and the formation of blue or greenish colour indicates the presence of tannins. ^[9]

Test for Saponins

Foam test: 0.5ml of the extract was shaken with water and the formation of persistent foam indicates the presence of saponins. ^[12]

Test for Sterols

Sulphuric acid (H₂SO₄) test: 0.5 ml of the extract was treated with ethanol and sulphuric acid (H₂SO₄) and the formation of violet, blue, green colour indicates the presence of sterols. ^[11]

Test for Phenols

Ferric chloride test: 0.5 ml of the extract was treated with 5% ferric chloride and the formation of deep blue or black or green colour indicates the presence of Phenols. ^[10]

Test for Glycosides

Keller killiani test: Test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added and observed for the formation of two layer. Lower reddish brown and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Proteins

Ninhydrin (acetone) test: 0.5 ml of the extract was treated with ninhydrin solution (ninhydrin dissolved in acetone) and the formation of purple colour indicates the presence of protein.

Test for carbohydrates

Fehling's test: 0.5g of the extract was dissolved in distilled water and filtrated. The filtrate was heated with 5ml of equal volumes of solution A and B. Formation of red precipitate of cuprous oxide was an indication of the presence of reducing sugars. ^[12]

Solution A: 0.1 M Copper (II) sulphate penta hydrate

Solution B: 0.1 M sodium potassium tartarate and 3 M NaOH

Test for Terpenoids

5ml of extract is mixed with 2ml of chloroform in a test tube. 3ml of the concentrated sulphuric acid (H₂SO₄) is carefully added to the mixture to form a layer. An inference with a reddish brown coloration is formed. ^[13]

Synthesis of copper nanoparticles using hydroalcohol extract of *Lawsonia inermis*

For synthesizing copper nanoparticles 10g of root powder weighed and dissolved in 100ml of hydroalcohol which was further subjected to hot hydroalcohol extraction and filtration. 270ml of CuSO₄ solution was taken and mixed with 30ml of the filtered root extract. It was then incubated for 24 hours in dark. After 24 hours of incubation the nanoparticles synthesized solution was heated for about an hour and added 2-3 drops of NaOH drop by drop. The solution was then subjected to centrifugation at 6000rpm for 10 minutes. The supernatant is discarded and the pellet was collected and made paste in watchglass to which few drops of petroleum ether was added. After drying it is scrapped of powder and stored in eppendorf tubes for further studies.

Characterization of Synthesis Nanoparticles

The characterization of nanoparticles is a branch of nano metrology that deals with the characterization or measurement of the physical and chemical properties of nanoparticles. Nanoparticles are unlike conventional chemicals in that their chemical composition and concentration are not sufficient metrics for a complete description, because they vary in other physical properties such as size, shape, surface properties, crystallinity and dispersion state. Nanoparticles are characterized for various purposes, including nano toxicology studies and exposure assessment in workplaces to assess their health and safety hazards, as well as manufacturing process control. There is a wide range of instrumentation to measure these properties, including microscopy and spectroscopy methods as well as particle counters. The characterization methods used here in the present study includes UV-spectroscopy, FTIR, and FESEM.

Uv-Visible Spectroscopic Analysis

For monitoring the signature of CuNPs, UV-Visible spectroscopy was used as a powerful tool for the characterization of colloidal particles. Noble metal particles are ideal candidates for the study with UV-VIS spectroscopy, while they exhibit strong surface plasmon resonance (SPR) in the visible region and are highly sensitive to the surface adaptation. The reduction of Cu⁺ ions was monitored by measuring the UV-VIS spectrum of the solution.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FT-IR analysis of CuNPs was carried out on a Shimadzu IR- Prestige-21 (Shimadzu Corp., Japan) in the diffuse reflectance mode operated at a resolution of 4cm⁻¹ in the range of 400-4000cm⁻¹ to evaluate the functional groups that might be involved in nanoparticles.

In-Vitro Anti-Cancer activity in Cell Line (A549):

HT-29 human colon cancer cell line was obtained from the National Center for Cell Science, Pune, India. It was cultured in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin (10,000 U/ml) in a 5% CO₂ humidified atmosphere at 37 °C. The cytotoxic potential of the metal oxides was assessed using the MTT assay.

3. RESULTS AND DISCUSSION

Qualitative analysis of root extract of *Lawsonia inermis*

Phytochemicals are secondary metabolites produced by all roots. The phytochemical research approach was considered effective in discovering bioactive profile of plants of therapeutic importance. The hydroalcohol extract of root contains alkaloids, flavanoids, tannis, phenols and terpenoids. In root extracts showed the presence of flavonoid, tannis, alkaloids in hydroalcohol extract (Table 1).^[14] Confirmed the presence of various components, such as carbohydrates, steroids, glycosides, saponins, terpenoids in *L. inermis*. A variety of plant ingredients with diverse structures are capable of promoting health benefits. These secondary metabolites are widely used in human therapy, veterinary, agriculture, scientific research and in countless other area.^[15]

Table:1 Phytochemical analysis of different extracts of root of *Lawsonia inermis*

S.no	List of the particulars	Aqueous	Ethanol	Chloroform	Petroleum ether	Hydro alcohol
1	Alkaloids	++	++	+	+	++
2	Flavonoids	++	++	+	+	++
3	Tannins	++	++	+	+	++
4	Saponins	++	+	+	+	++
5	Steroids	-	+	-	-	++
6	Phenols	-	+	-	-	+
7	Glycosides	-	+	-	-	+
8	Protein and amino acids	-	+	-	-	++
9	Carbohydrates	+	++	-	+	++
10	Terpenoids	+	+	-	-	++
11	Coumarin	-	-	-	-	-
12	Cardioglycosides	+	+	-	-	++
13	Reducing agent	-	+	-	-	++
14	Anthraquinone	+	+	-	-	++
15	Phlobatanins	-	-	-	-	+

Synthesis of Copper Nanoparticle from root *Lawsonia inermis*

The copper nanoparticles are synthesized from the *Lawsonia inermis*, was visually observed from the colour change. The colour of the *Lawsonia inermis* and copper nitrate was changed from blue colour to greenish brown colour, the change in colour was stable and the precipitation were found to settle at the bottom of the solution.



Fig 1: The colour change in the synthesis of copper nanoparticles.

Characterization of Copper Nanoparticles

The copper nanoparticles was characterized by UV- Visible spectroscopy and Fourier Transformed Infrared Spectroscopy (FT-IR) analysis.

UV- Visible spectroscopy

A facile and green route for the synthesis of copper nanoparticles (CuNPs) has been achieved using green tea extract as a reducing, capping and stabilizing agent. UV-visible spectra gave surface plasmon resonance at 560nm. The CuNPs were characterized using various techniques.

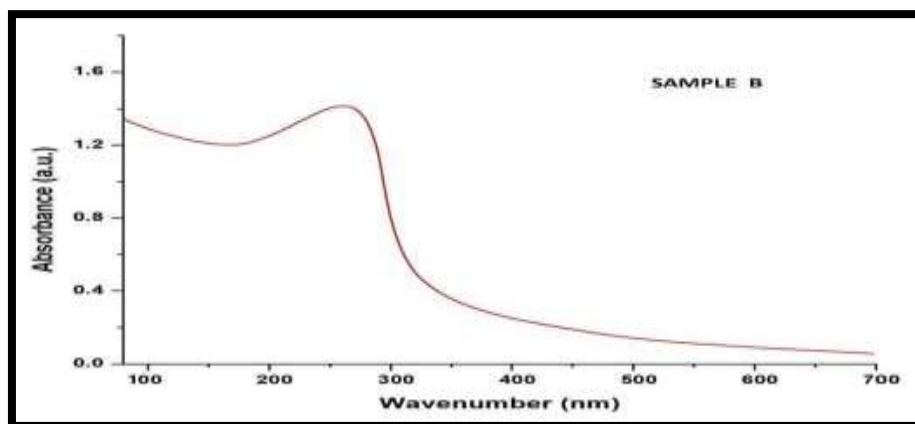


Fig 2 : UV-spectroscopy analysis of CuNPs.

The size of CuNPs was about 300nm. The present study mainly aimed at the synthesis of copper oxide nanoparticles of varied size by green synthetic approach. At a wave length of 300nm, the optimum parameter required for CuNPs synthesis. UV-Visible spectroscopy provides only the preliminary information of formation of copper nanoparticles. Therefore, the synthesized copper nanoparticles were characterized using the other advanced techniques.

Fourier Transformed Infrared Spectroscopy (FT-IR)

To understand the involvement of functional groups and the interactions between the metal particles and biochemical, FT-IR plays an important tool ^[15]. Copper has excellent conductivity. Due to relatively low cost, this metals plays an significant role in modern electronic circuit. Because of its excellent conductivity, catalytic behavior, good comparability and surface enhanced Raman scattering activity. Cu nanoparticles have drawn the attention of scientists to be used as essential component in the future Nano-devices.^[16]

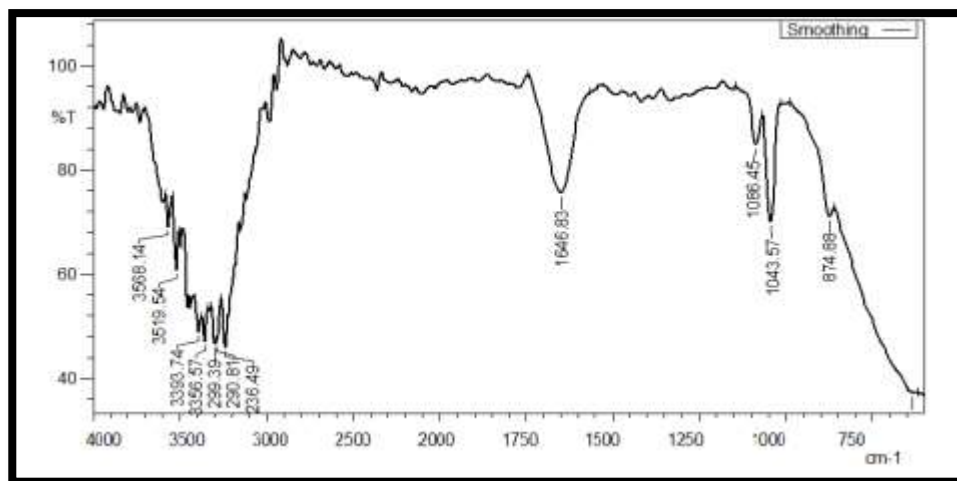


Fig 3: FT-IR Pattern of synthesized copper nanoparticles in the hydroalcohol extract of *L. inermis*

Cu nanowires are used in Nano electronics and have application possibilities for magnetic devices, Nano sensors, electron emitters and other electronic applications. Cu nanoparticles have been explored to be used in Nano probes in medicines and bio-analytical areas. High temperature superconductivity materials are mostly prepared from CuO based compounds ^[17] similarly semiconducting anti-Ferro magnetic materials are also synthesized from Cu. Nanoparticles are a class of materials with properties which differ from their characteristics and find the use in different areas such as electronic, magnetics, pharamechutial, cosmetic energy, catalytic and materials applications.

***In-Vito* Anti-Cancer Activity in Colon Cell Lines (Ht-29)**

The reasons why cancer cells are addicted to aerobic glycolysis and the underlying molecular mechanisms still remain controversial. Mitochondrial dysfunction caused by mitochondrial DNA mutations, oncogenic signals, and ROS stress may be an important event that forces cancer cells to be more rely on the glycolytic pathway for energy production and for generation of metabolic intermediates for biogenesis. Glycolysis may be still highly active even in cancer cells with competent mitochondria function and activate oxidative phosphorylation, and thus blocking a single energy metabolic pathway might not be effect in killing cancer cells^[18] The previous study shows, *Acalypha indica*-mediated synthesized copper oxide nanoparticles exerted cytotoxic effects on the MCF-7 cancer cell line.^[19]

The *in-vitro* anti-cancer activity in HT-29 colon cell lines was evaluated by MTT assay. The MTT assay is used to measure the cellular metabolic activity as an indicator of the cell viability, proliferation and cytotoxicity. The result of the MTT is shown in the Figure 4 and the results of cell viability and the cytotoxicity are given in the table 2. The cell viability decreases while the cytotoxicity increases along with the increase in the concentration of the sample which indicates that it has potential cytotoxic effect against the colon cancer cell lines (HT-29).

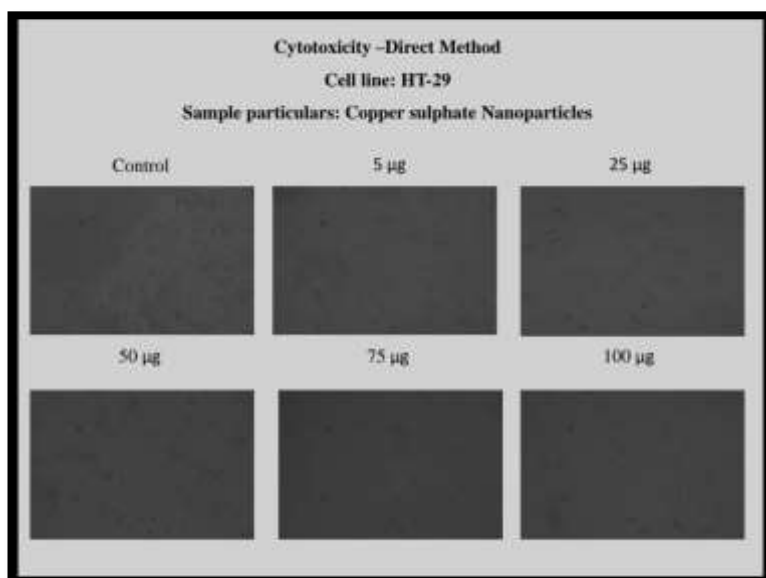


Fig 4: Images of MTT assay for evaluation of anti-cancer activity.

Table 2: Result of cell viability and the cytotoxicity according to the sample concentration.

SAMPLE CONCENTRATION	CYTOTOXICITY	CELL VIABILITY
5µ	75.8	24.2
25µ	77.3	22.7
50µ	88.9	11.1
75µ	89.3	10.7
100µ	91.7	8.3

The pervious results revealed that anti-cancer potential of *M. azedarach*, *P. benthamii*, *B. pacumbis*, and *M. koenigii* extracts against HeLa, Hep3B, and HCT116 cell lines among six analyzed plant extract. However, *A. racemosus* and *T. linearis* showed relatively lower inhibitory activities against the analyzed cancer cell lines and also found toxic to the normal cell. Although studies on anticancer potential of different fractions and pure isolates of *M. azedarach*, *A. racemosus*, *T. linearis*, and *M. koenigii* have been already carried out in different cell lines, no detail investigations were carried out for these plant extracts against examined cancer cell lines. Previous studies on *M. azedarach* extract against human urinary bladder carcinoma cells and human breast cancer cells possess no significant inhibition. However, some steroids isolated from *M. azedarach* showed good anticancer potential with low cytotoxicity against human lung cancer cell lines and the human glioma cancer cell line.^[20]

Plant species evaluated for its potential biological activities, *L. inermis* root revealed the good anti-cancer. However, *L. inermis* root shows remarkable wound healing and anti-inflammatory activities with no toxicity. Although, other plant species possess antiproliferative activity, the toxicity was high for the consideration of anticancer therapeutic. Our result opens up the possibility in the future to identify the potential therapeutic agents from *L. inermis* root for the development of herbal-based medicine.

CONCLUSION

In the present study, the hydroalcohol root extracts of *Lawsonia inermis* were subjected to anticancer studies which revealed that the high free radical scavenging activity and showed maximum inhibitory activity on human pathogens and the copper nanoparticles was synthesised with the *Lawsonia inermis* and its biological properties was evaluated. The results show that the root extracts of *Lawsonia inermis* can be used as one of the unconventional resources for green synthesis of CuO nanoparticles. Analytical approaches such as the UV-Visible and FT-IR used to determine the nature of synthesized CuO nanoparticles. The anticancer activity of CuONPs that have been synthesized from the root extracts of *Lawsonia inermis* was demonstrated in a human colon cancer potential inhibition by using MTT assay. The use of nano-sized CuO based drug designing molecules is largely responsible for the novel anti-cancer drug's superior efficacy and decreased toxicity.

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