Areca Catechu Leaf Extract-Mediated Synthesis of Stabilized Silver Doped-Copper Oxide Nanoparticles and its Biomedical Applications

Shwetha UR

GM Institute of Technology

Virupaxappa S. Betageri

GM Institute of Technology

Latha M.S.

R L Science Institute

Ravindra Veerapur

Malawi Institute of Technology

Chandan Shivamallu

JSS Academy of Higher Education and Research

Shiva Prasad Kollur (✉ shivachemist@gmail.com)

Amrita School of Arts and Sciences <https://orcid.org/0000-0003-3914-3933>

Research Article

Keywords: Green synthesis, Ag-CuO NPs, Anticancer, Antidiabetic.

Posted Date: May 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-517512/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The current research work gives an insight into the green synthesis of metallic nanoparticle using medicinally important plant, which has gained increasing applications in therapeutics and medicinal science. Herein, we describe an eco-friendly method to synthesize Ag- CuO nanoparticles using *Areca catechu* leaf extract via solution combustion method. Crystalline structure, size and surface morphology of the synthesized nanoparticles were confirmed by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), Transmission Electron Microscopy (TEM) and Fourier Transformer Infrared Spectroscopy (FTIR). XRD patterns of the as-prepared Ag-CuO NPs revealed that they possess monoclinic crystalline structure. The morphology and the elemental composition was found by SEM-EDS and TEM analysis, which revealed the nano-spherical shape of Ag-CuO NPs. Further, the Ag-CuO NPs were tested for Antidiabetic activity by using Yeast cell model and analyze inhibition, which showed promising antidiabetic activity. In addition, the as-prepared material displayed significant anticancer activity against MCF-7 cancer cell line.

1. Introduction

Nanoparticles are the cornerstone of nanotechnology and nanoscience which have enormous potential and functional ability in various fields. The pharmaceutical world is looking forward for alternative drugs with nano size and should have targeted delivery. Thus, young researchers are interested to explore medicinally important molecules and nanotechnology seems to be the result of that curiosity. Nanotechnology is one of the emerging sciences which deals with synthesis, fabrication and manipulation of materials with dimensions in the small range of 1-100 nm. In India, Ayurveda is an ancient medicine system which deals with the different formulations which are suggestive about the nano size range [1, 2]. Thus, an era of nanomedicine is rapidly developing and promising field that immensely benefit through the best use of medicinal plants to synthesizing metallic nanoparticles with high therapeutic value. [3].

Among various noble metal nanoparticles, silver nanoparticles are being studied extensively among noble metal nanoparticles by scientists due to their distinctive physico-chemical properties like chemical stability, magnetic and inimitable optoelectronic characteristics [4, 5]. These distinct properties of silver nanoparticles have been used for a wide range of applications including biotechnology, sensors, catalysis, electronics, superconductors, and biomedical fields for cancer treatment, diabetics, antibacterial agent etc [6–8]. In spite of innumerable applications of monometallic nanoparticles, bimetallic nanoparticles have possessed enhanced properties due to enhanced reactivity of constituent metals [9]. Remarkable properties of copper nanoparticles have grabbed attention due to its advanced applications in biology and nanomedicine where they are often used as fungicides, antifouling and antibacterial agent, water purifiers [10, 11]. Among the various semiconductors metal oxides, CuO belongs to p-type semiconductor having a band gap of 12 eV. Nowadays, CuO NPs is of great interest owing to its wide application in lithium electrode material, solar cells, electronics, gas sensing and catalysis. Further, it is an inherent element that are enrich in the planet surface, being nontoxic and economical production

processing [12–14]. Exploiting the potential of medicinal plants have diverted researcher attention to the green route because of the current therapeutic approaches have owing to its eco-friendly, most reliable, and sustainable [15]. The biomolecules and secondary metabolites present in the plant extract act as a reducing and capping agent helps in the easy reduction of metal ions into nanoparticles.

Diabetes is a metabolic disease which is characterized with high blood glucose or insulin intolerance. Modern life style, food habits and stress are common reasons for this disorder which affecting people of all age groups. [16]. Cancer is one of the major challenges to human health as 18.1 million new cancer cases were diagnosed in 2018 [17]. Breast cancer is the most commonly occurring cancer in the women worldwide, and continued to increase with passage of the time and credited with second leading cause of the death in women if compared with other types of cancer.

In this work, *Areca catechu* leaf extract was used as reducing and capping agents for the bio-synthesis of Ag-CuO nanoparticles. *Areca* is a species of palm which grows in much of the tropical Pacific, Asia and some part of east Africa. The principal constituents of *Areca catechu* are flavonoids, polyphenols and alkaloids such as arecaine, guavacine and arecoline [18].

2. Materials And Methods

All the chemicals and regents (Analytical grade) were procured from SD Fine and Himedia Laboratory Pvt. Ltd., Mumbai, India, and used as received. The size and shape of as-prepared Ag-CuO NPs was measured on Transmission Electron Microscopy (TEM-1011, JEOL, Tokyo, Japan). The surface morphology and elemental composition of the obtained material was measured using Scanning Electron Microscope with Energy dispersive X-ray Analysis (Hitachi S3400n, Tokyo, Japan). X-ray diffraction pattern was recorded on a PANalytical X'Pert-PRO (Rigaku Smart Lab). FT-IR spectrum of the as-prepared material was recorded using a Perkin Elmer FT-IR spectrometer in the frequency range $4000\text{--}350\text{ cm}^{-1}$ (Paragon-1000-FTIR, Buckinghamshire, UK).

2.1. Preparation of Areca leaves extract

Fresh leaves of *Areca catechu* plant were collected from Agasanakatte village, Davanagere district, Karnataka and leaves were cleaned with distilled water, dried under sunlight and powdered. 20 g of leaves powder dissolved in 100 ml of deionized water, kept for boiling at 60°C for 1 h until the colour gets changed to dark green. Prepared leaf extract was then kept for cooling at room temperature. Obtained extract was filtered twice with whattman's filter paper no.1 and filtrate was stored at 4°C for further use.

2.2. Synthesis of Ag-CuO NPs

Ag-CuO NPs was synthesised through solution combustion method [19]. Here precursors were Copper nitrate hexahydrate and silver nitrate and *Areca catechu* leaf extract as a fuel. $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (95%) and AgNO_3 (5%) were taken in a beaker containing 10 ml of the *Areca catechu* leaf extract. Obtained solution is constantly mixed thoroughly to get homogeneous solution. This is then placed in a preheated muffle

furnace (600°C), where vigorous combustion reaction takes place that lead to formation of Ag-CuO NPs. As-obtained NPs were stored in airtight container for further use [20].

2.3. Antidiabetic studies

2.3.1. Preparation of yeast suspension

Experiment was performed by taking 1 g of the commercial Baker's yeast. Yeast suspended in distilled water and washed by repetitive centrifugation at 3000 RPM about 5 minutes until the clear supernatant fluids were obtained and a 10% (v/v) suspension was prepared in distilled water. To the various concentrations of green synthesized Ag-CuO NPs (50–250 µg/mL) was added to 1 mL of 5 mM glucose solution and whole mixture was incubated for 10 min at 37°C. The reaction was started by adding 100 µL of yeast suspension to glucose solution containing Ag-CuO NPs, then it is vortexed and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged at 2,500 RPM for 5 min. Later the increase percentage of glucose uptake concentration by yeast cells was determined by UV-Visible spectrophotometer at 520 nm. Distilled water was used as a blank. Here Metranidazole was taken as standard reference drug for analysis [21]. The percentage increase in glucose uptake by yeast cells was calculated using the below formula:

$$\text{Increase in glucose uptake \%} = \frac{A_{(\text{sample})} - A_{(\text{control})}}{A_{(\text{sample})}} \times 100$$

2.4 Amylase activity

The antidiabetic activity was investigated by using α -amylase inhibition assay model [22]. In brief, to a fresh 0.5 ml of different concentrations of Ag-CuO NPs (100, 200, 300, 400 and 500 µg/ml) was mixed 1 ml of PBS solution and 200 µl of 0.5 ml of α -amylase was added followed by 200 µl of 5 mg/ml starch solution and incubated at room temperature for 10 minutes. Here starch with amylase and without α -amylase was taken as a control. Finally, the reaction was terminated by adding 400 µl of dinitrosalicylic acid and placed in a boiling water bath for 5 min, later it is cooled. The absorbance was determined at 540 nm (Labman UV Visible Spectrophotometer). Metformin is used as a standard reference here. The percentage of inhibition of α -amylase was calculated using the following equation:

$$\% \text{ of } \alpha - \text{amylase inhibition} = \frac{A_{(\text{control})} - A_{(\text{sample})}}{A_{(\text{control})}} \times 100$$

2.5 In vitro cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed for synthesized Ag doped CuO NPs against MCF-7 cancer cells. Breast cancer cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) liquid medium with the aid of 96-well microtiter and incubated in 5% CO₂ atmosphere for 24 h at 37 °C. Cells were then washed and treated with different concentrations of the sample (20 µg/ml to 100 µg/ml), again incubated at 37 °C in a humidified 5% CO₂ incubator for 24 h.

After the incubation period, 10% MTT (200 μ l) was added into each well and the cells incubated for 3 h. The absorbance for each well was measured at 570 nm using a microplate reader. The IC₅₀ values were calculated using dose response curve [23].

3. Result And Discussion

3.1. XRD analysis

XRD studies is a key tool to investigate crystalline structures and phase purity and the obtained XRD patterns are delineated in Fig. 1. For Ag-CuO nanoparticles, the obtained major diffraction peaks at 2θ values 32.11°, 35.50°, 38.08°, 44.29°, 64.47° and 77.37° are observed, which indexed to the (110), (002), (111), (200), (220) and (311) planes. The sharp intense peaks at (200), (220) and (311) is identified presence of Ag. All the diffraction peaks are in full agreement to monoclinic CuO (JCPDS no. 80-1917) and cubic Ag (JCPDS no. 04-0783), which indicates that NPs are composed of pure Ag-CuO. The average particle size has been determined using Debye–Scherrer formula and was found to be 29.18 nm [24].

3.2. Structural morphology

The crystal structure, size distribution and surface morphology of the synthesized Ag-CuO NPs were elucidated using SEM, EDX and HR-TEM. From SEM images it is clear that the NPs were have spherical morphology with slight agglomeration (Fig. 2). From Fig. 3 presence of Cu, O and Ag elements was proven by EDX results. Further TEM images showed the formation of monoclinic crystal structure particles. The presence of diffraction rings in SAED patterns supports the existence of monoclinic crystal phase.

3.3. Glucose uptake by yeast cells

Different concentrations of test samples are subjected to *in vitro* glucose uptake assay employing yeast as model. The percentage of glucose uptake in yeast cells by the test samples were compared with standard drug metronidazole. There was concentration dependent increase in percentage of glucose uptake with increasing in concentration of Ag-CuO NPs shown in Table 1. Ag-CuO nanoparticle exhibited highest percentage of glucose uptake i.e. 52.7407 ± 0.0236 , which was comparable to the standard i.e. 88.459 ± 0.0002 at 250 μ g concentration. In yeast, glucose transport takes place through facilitated diffusion. Type 2 diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. The rate of glucose transport across cell membrane in yeast cells was presented in Fig. 5. It is reported that in yeast cells, glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast by facilitated diffusion process through specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose. The Ag-CuO NPs exhibited significantly higher activity at all glucose concentrations. Results also indicated that green synthesized Ag-CuO NPs had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug [24].

Table 1
Antidiabetic activity of Ag-CuO nanoparticles.

Sl. No	Concentration (µg/ml)	Percentage of glucose uptake by test sample	Percentage of glucose uptake by standard drug
1	50	7.9170 ± 0.01389	22.675 ± 0.0003
2	100	21.6511 ± 0.0535	51.669 ± 0.0001
3	150	39.4696 ± 0.0670	59.908 ± 0.0001
4	200	51.1382 ± 0.0126	78.284 ± 0.0011
5	250	52.7407 ± 0.0236	88.459 ± 0.0002
Results are expressed as mean ± standard error			

3.4. Amylase assay report

The α-amylase inhibitor effectiveness of Ag-CuO nanoparticle was compared with standard drug Metformin (Table 2). The Ag-CuO NPs showed a percentage inhibition of 2.8841 and 3.7817 at 20 µg/ml and 100 µg/ml, respectively. Standard drug Metformin showed inhibitory effects on the α-amylase activity with an IC₅₀ value 232.12 µg/mL. As-prepared Ag-CuO NPs from *Areca catechu* leaves exhibited α-amylase inhibitory activity with an IC₅₀ values 2087.19 µg/mL. As the result, as-synthesized Ag-CuO nanoparticle showed substantial antidiabetic activity when compared to Metformin [25].

Table 2
Percentage of α-amylase Inhibition by sample and standard drug.

Sl. No	Concentrations µg/ml	% Inhibition by sample Ag-CuO nanoparticle	% Inhibition by standard drug Metformin
1	20	2.884155098	5.08616
2	40	0.466730493	8.87984
3	60	2.333652465	13.34370
4	80	3.350885591	16.51507
5	100	3.781713739	22.59454

3.5. Anticancer activity

Cytotoxicity of the green synthesized Ag-CuO nanoparticle was evaluated against breast cancer cell lines (MCF-7). In this study, cell line containing various concentrations of Ag-CuO NPs (20, 40, 60, 80 and 100 µg/ml) were taken after incubation for 24 h. The percentage of cell viability was calculated from the

optical density (OD) values of MTT assay as shown in Fig. 6. The synthesized Ag-CuO NPs exhibited minimum inhibition at an initial concentration at 20 µg/ml and maximum at 100 µg/ml (Fig. 7). Subsequently, as the concentration of Ag-CuO NPs increases the cancer cell viability decreases. Hence the synthesized nanoparticles showed a significant cytotoxicity against MCF-7 cells. Further, the IC₅₀ values for the cells were 11.217 µg/ml, which clearly shows that Ag-CuO NPs has high efficacy against MCF-7 cancer cell lines [26].

4. Conclusion

In this study we have reported facile synthesis of Ag doped CuO nanoparticles by using *Areca catechu* leaf extract via solution combustion method. Therapeutic values of medicinal plants are exploited for the benefit of mankind. From the characterization studies, it is evident that the green synthesized Ag-CuO NPs were spherical in shape with slight agglomeration and having average particle size between 10 and 40 nm. Further, *in vitro* antidiabetic assays carried out by using amylase assay and yeast cell model which showed higher hypoglycaemic effect of Ag-CuO NPs. Obtained result has proved significant antidiabetic activity of Ag-CuO NPs. Moreover, Ag-CuO NPs has potent anticancer activity against MCF-7 cancer cell line. Hence, these results encourage that, plant mediated NPs can be used in various biomedical applications.

Declarations

Acknowledgments: Authors thank the Director, Indian Institute of Science, Bengaluru, India for analytical facilities. KSP is grateful to the Director, Amrita Vishwa Vidyapeetham, Mysuru campus for infrastructure support. CS acknowledge the support and infrastructure provided by the JSS Academy of Higher Education and Research (JSSAHER), Mysuru, India. The authors are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

References

- [1] Pal, D.; Sahu, C.K.; Halder, A. J. Adv. Pharm. Technol. Res. 2014, 5, 4-12.
- [2] Sharma, R.; Prajapati, P.K. J. Pharm. Bioallied Sci. 2016, 8, 80-81.
- [3] Muhammad Mailafiya, M.; Abubakar, K.; Danmaigoro, A.; Musa Chiroma, S.; Bin Abdul Rahim, E.; Aris Mohd Moklas, M.; Abu Bakar Zakaria, Z. Appl. Sci. 2019, 9, 2897.
- [4] Crumbliss, A. L.; Perine, S. C.; Stonehuerner, J.; Tubergen, K. R.; Zhao, J.; Henkens, R. W.; O'Daly, J. P. Biotechnol Bioeng, 1992, 40, 483-490.

- [5] Muthushanmugam, M.; Subramanian, P.; Manoharan, V.; Baskaran, K.; Sonaimuthu, M.; Manikandan, M.; Tabarsa, M.; You, S.; Marimuthu, P. N. J. Drug Del. Sci. Tech. 2019, 54, 101345.
- [6] Yurovaa, N. S.; Markinaa, N. E.; Pozharova, M. V.; Zakharevichb, A. M.; Rusanovaa, T. Y.; Markin, A. V. Colloids Surf., A. 2016, 495, 169-175.
- [7] Rajan, A.; Vilas, V.; Philip, D. J. Mol. Liq. 2015, 207, 231-236.
- [8] Bhattacharya, R.; Mukherjee, P. Adv. drug Del. Rev. 2008, 60, 1289e1306.
- [9] Zaleska-Medynska, A.; Marchelek, M.; Diak, M.; Grabowska, E. Adv. Colloid Interface Sci. 2016, 229, 80-107.
- [10] Kalatehjari, P.; Yousefian, M.; Ali Khalilzadeh, M. J. Aquat. Res. 2015, 41, 303-306.
- [11] Kruka, T.; Szczepanowicz, K.; Stefanska, J.; Sochaa, R. P.; Warszynski, P. Colloids Surf. B. 2015, 128, 17-22.
- [12] Zou, X.; Fan, H.; Tian, Y.; Zhang, M.; Yau, X. Dalton Trans. 2015, 44, 7811–7821.
- [13] Mishina, E. D.; Nagai, K.; Nakabayashi, S. Nano Lett. 2001, 1, 401–404.
- [14] Karunakaran, C.; Manikandan, G.; Gomathisankar, P. J. Alloy. Compd. 2013, 580, 570–577.
- [15] Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A. J. Clin. 2018, 68, 394–424.
- [16] Aruoma, O. I. Free Radical Res 1999; 30: 419-427.
- [17] Koehn, F. E.; Carter, G.T. Nat. Rev. Drug Discov. 2005, 4, 206–220.
- [18] Kotepui, M. Oncol. 2016, 20, 13.
- [19] Shwetha, U. R.; Rajith Kumar, C. R.; Kiran, M. S.; Virupaxappa, S. B.; Latha, M. S.; Ravindra Veerapur; Ghada Lamraoui; Abdulaziz A. A.; Abdallah M. E.; Asad Syed; Chandan, S.; Shiva Prasad, K. Molecules, 2021, 26, 2448.
- [20] Rajith Kumar, C.R.; Betageri, V. S.; Nagaraju, G.; Pujar, G. H.; Suma, B. P.; Latha, M. S. J. Inorg. Organomet. Polym. Mater. 2020, 30, 1–11.
- [21] Tarling, C. A.; Woods, K.; Zhang, R.; Brastianos, H. C.; Brayer, G. D.; Andersen, R. J. Chem Bio Chem. 2008, 9, 433-438.
- [22] Nabanita Patra; Dattatreya Kar; Abhisek Pal; Anindita Behera. Adv. Nat. Sci.: Nanosci. Nanotechnol. 2018, 9, 035001.

[23] Shwetha, U. R.; Latha, M. S.; Rajith Kumar, C. R.; Kiran, M. S.; Virupaxappa S. Betageri. J. Inorg. Organomet. Polym. Mat. 2020, 30, 4876-4883.

[24] Jadhav, M.; Kulkarni, S.; Raikar, P.; Barretto, D. A.; Vootla, S. K.; Raikar, U.S. New J. Chem., 2017, 41, 9513-9120,

[25] Punitha, R.; Manoharan, S. J. Ethno. Pharmacol. 2006, 105: 39-46.

[26] Vani, M.; Vasavi, T.; Uma Maheswari Devi, P. Asian J Pharm Clin Res, 2018, 11, 150-153.

Figures

Figure 1

X-ray diffraction pattern of as-prepared Ag-CuO NPs.

Figure 2

SEM images of green synthesized Ag-CuO NPs.

Figure 3

EDX spectrum of green synthesized Ag-CuO NPs.

Figure 4

(a-c) TEM manographs and (d) SAED pattern of as-prepared Ag-CuO NPs.

Figure 5

The percentage of glucose transport across cell membrane in yeast cells as compared with standard drug.

Figure 6

Anticancer activity with respect to the standard, control for different concentrations of Ag-CuO NPs against MCF-7 cancer cell lines.

Figure 7

Cell lines (MCF-7) viability treated with different concentration of as-prepared Ag-CuO NPs.