Research and PG Department of Botany
ST.THOMAS COLLEGE *(Autonomous)*
THRISSUR - 680 001, KERALA, INDIA
(Affiliated to University of Calicut, NAAC with 'A' Grade and College with Potential for Excellence)
Estd. 1889
Web:- http://stthomas.ac.in Email:- stcthrissur@gmail.com
Phone:- +91 487 2420435 Fax:- +91 487 2421510

Date: 06/05/2022

CERTIFICATE

I hereby certify that, this is the revised version of the thesis entitled ***"Green synthesis of Silver Nanoparticles using Curcuma Rhizomes: Characterization and Application"*** submitted by **Ms. Aiswariya K S,** under my guidance after incorporating the necessary corrections / suggestions made by the adjudicators. The content of the CD is the same as in the hard copy.

Dr. Vimala Jose

(Research Guide)

HEAD OF THE DEPARTMENT
Department Of Botany
St. Thomas' College(Autonomous)
Thrissur, Kerala-680001

Estd. 1889

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CERTIFICATE

This is to certify that the thesis entitled ***"Green synthesis of Silver Nanoparticles using Curcuma Rhizomes: Characterization and Application"*** is an authentic record of original research carried out by **Ms. Aiswariya K S** under my supervision in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Botany of University of Calicut and further that no part thereof has been presented before for any other degree.

ST. THOMAS' COLLEGE DEPARTMENT OF BOTANY THRISSUR

Dr. Vimala Jose

(Research Guide)

HEAD OF THE DEPARTMENT
Department Of Botany
St. Thomas' College(Autonomous)
Thrissur, Kerala-680001

Declaration

I hereby declare that the thesis entitled ***"Green synthesis of Silver Nanoparticles using Curcuma Rhizomes: Characterization and Application"****, submitted to the University of Calicut in partial fulfillment of the requirement for the award of the Degree of Doctor of Philosophy in Botany is a bonafied research work done by me under the supervision of* ***Dr. Vimala Jose****, Assistant Professor, Research and Post graduate Department of Botany, St. Thomas College (Autonomous), Thrissur.*

I also declare that the material presented in this thesis is original and does not form the basis for the award of any other degree, diploma or other similar titles of any other university.

Date: 06/05/2022

AISWARIYA K S

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With heartfelt gratitude

AISWARIYA K S

Dedicated to

The Almighty

Preface

Nanotechnology refers to the wide range of technologies and applications that involve the use of particles ranging from a few nanometers to hundreds of nanometers in diameter. Nanoparticles (NPs) have revolutionized the fields of environmental remediation, medicine, material science, chemistry and engineering. They have been exploited in catalysis, sensor technology, imaging, cancer treatments and site specific drug delivery because of their characteristic high surface to volume ratio compared to their bulk counterparts. But the physicochemical processes involved in the synthesis of metallic nanoparticles involve the use of toxic solvents, posing a serious threat to the environment. Their efficacy, on the other hand, is restricted by their limited hydrophilicity and stability. Therefore, in recent times, the use of natural entities such as microbes, parasites, yeast, seaweeds and plants as basic hotspot for the synthesis of metal nanoparticles has gained considerable interest among researchers. Plants are enriched with bioactive molecules which are unique in structure and function justifying their role in pharmaceutical, biomedical, nutraceutical, cosmeceutical, and chemical industries. Hence, the plants continue to be a critical source of present-day drugs Natural products are frequently viewed as vulnerable medication candidates due to their high dosage requirements and frequent administration.

Despite the advantages of the biomolecules, the exploitation of the plant resources remains in a nascent stage. Consequently, during the recent years, synthesis of plant based functional nanoparticles has evolved as a potential area of investigation among the scientific community. Besides, the minimal efforts involved in synthesis, the plant material integrated nanoparticles are biocompatible and biodegradable. Moreover, the bioactive molecules act as reducing and stabilizing agents in the formation of metal nanoparticles. Therefore, the green synthesized nanoparticles have been explored in the field of nanomedicine and nanoremediation.

The main goal of this research is to produce silver nanoparticles (AgNPs) using aqueous extracts of *Curcuma zanthorrhiza* Roxb. (CZ) and *Curcuma aromatica* Salisb. (CA), as well as to evaluate its diverse catalytic, antimicrobial, antioxidant, *in vitro* and *in vivo* anticancer properties. The toxicity evaluation of silver nanoparticles was also performed.

To establish the synthesis of silver nanoparticles, UV-Visible spectroscopy experiments were carried out. FTIR investigations have been carried out to ensure the nature off phytochemicals involved in the reduction and stability of nanoparticles. The crystalline nature of silver nanoparticles was investigated using X-ray diffraction techniques. The size and shape of the nanoparticles were studied using HR-TEM and FESEM. Energy dispersive X-ray (EDX) analysis was used to characterize the elemental properties of nanoparticles. DLS was used to investigate the hydrodynamic size of the synthesized silver nanoparticles, while BET analysis was used to determine its surface area. HR-LCMS analysis of nanoparticles was done to ascertain the chemical nature of biomolecules that capped over the AgNPs.

In the field of catalysis, promising new possibilities based on nanotechnology methods are developing. Catalyst synthesis that is efficient, size regulated, and cost effective is thus extremely important. Nanoparticles have been found to offer novel catalytic features, such as increased reactivity and selectivity, when compared to their bulk counterparts. The catalytic efficiency of synthesized nanoparticles (CZAgNPs and CAAgNPs) in the degradation of ionic dyes (malachite green and coomassie brilliant blue), by photons (sunlight and UV light irradiation) was investigated. The catalytic property of nanoparticles were further validated by analyzing the degradation of azo dyes (Orange G, Methyl Orange, Eriochrome Black T, and Congo Red) using sodium borohydride as a reductant. UV-Visible spectral analysis has been used to investigate the reduction patterns of various dyes. HR LCMS was further used to ascertain degradation products.

Metal nanoparticles have antimicrobial properties that have been used to treat a number of different infections. Nanoparticles can bind to microorganisms more effectively due to their small size and large surface area. In light of the potential applications of silver nanoparticles in many biomedical domains the antibacterial properties of synthesized CZAgNPs and CAAgNPs were examined using the disc diffusion method and the broth microdilution assay. The antifungal property of nanoparticles was investigated by the poisoned food technique. *In silico* molecular docking experiments were done using the tool Auto Dock 4.2. Here rigid protein flexible ligand docking was employed to study the interactions.

The biomedical applications of nanoparticles are becoming more widespread. However, there are not many studies regarding the influence of these nanoparticles in living cells or as biochemical indicators. It is therefore vital to encourage research into the toxicity and biochemical evaluations of silver nanoparticles. In the present investigations, the antioxidant potentials of plant extracts and AgNP synthesized from them have been assessed using an *in vitro* DPPH assay.

The trypan blue exclusion method in DLA and EAC cell lines, as well as the MTT assay in MCF - 7 cell lines, was used to assess the short-term *in vitro* cytotoxicity of plant extracts and the as synthesized silver nanoparticles. To further substantiate the cytotoxic properties of silver nanoparticles, *in silico* molecular docking investigations were conducted. The MCF - 7 proteins - Human tyrosine protein kinase C- SRC (PDB ID: 2SRC) and CDK 2 with EGFR inhibitor compound 8 (PDB ID: 4RJ3) were selected and was docked against the biomolecules bound on the as synthesized silver nanoparticles. The protein ligand bound complex was visualized using Pymol tool.

The safety evaluation of the synthesized nanoparticles has been conducted *in vivo* in Swiss albino male and female mice. Oxidative stress is one of the most important elements in the development of chronic and degenerative diseases such as ageing, cancer, and immunological suppression. Phytochemicals with antioxidant activity can be found in a variety of plants. Plant-based antioxidants are regarded as an essential source of therapeutic agents in comparison to current medications because of their low cost, ease of availability, and lack of side effects. Antioxidants can protect the body from the oxidative damage caused by free radicals. Antioxidants function as oxygen scavengers or react with free radicals to slow down the oxidation process. The protective impact of green synthesized silver nanoparticles on Swiss albino mice intoxicated with sodium fluoride was investigated.

The potential of silver nanoparticles in inhibiting the rapid growth and proliferation of both DLA and EAC tumour models was investigated in this study. The entire work is summarised in the section Summary and Conclusion. The possible application of biogenic AgNPs for prospective nanomedicine and nanocatalysts is discussed. The significance of the work along with its environmental friendliness and future scope is also discussed.

Abbreviations

NP	Nanoparticle
CZ	*Curcuma zanthorrhiza*
CA	*Curcuma aromatica*
DPPH	2,2-diphenyl -1-picrylhydrazyl
DLA	Dalton's Lymphoma Ascites
EAC	Ehrlich Ascites Carcinoma
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl- tetrazolium bromide
MCF - 7	Michigan Cancer Foundation-7
ROS	Reactive Oxygen Species
CADD	Computer Aided Drug Design
PDB	Protein Data Bank
DAP	Diaminopimelate
EGFR	Epidermal Growth Factor Receptor
FCR	Follin's Ciocalteau Reagent
OD	Optical Density
BJH	Barrett Joyner Halenda
MG	Malachite Green
CBB	Coomassie Brilliant Blue
OG	Orange G
CR	Congo Red
EBT	Eriochrome Black T
MO	Methyl Orange
ZOI	Zone of Inhibition
LB	Luria Bertani broth
MIC	Minimum Inhibitory Concentation

PDA	Potato Dextrose Agar
OECD	Organisation for Economic Co-operation and Development
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very low Density Lipoprotein
SGPT	Serum Glutamate Pyruvate Transaminase
SGOT	Serum Glutamate Oxaloacetic Transaminase
ALP	Alkaline phosphatase
CAT	Catalase
SOD	Superoxide dismutase
GSH	Reduced glutathione
GST	Glutathione - s- transferase
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
SPR	Surface Plasmon Resonance

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