5. Summary and Conclusion

Plants are enriched with bioactive compounds that are structurally and functionally unique, justifying their use in the pharmaceutical, biomedical, nutraceutical, cosmeceutical, and chemical sectors. As a result, plants are still an important source of modern medications. Plant derived bioactive molecules have the capacity to interact with a number of different chemicals. Consequently, during the recent years, synthesis of plant based functional nanoparticles has evolved as a potential area of investigation among the scientific community. Silver nanoparticles, which are among the noble metal nanoparticles, have become the subject of recent research due to their intriguing features and wide range of applications in a variety of fields. The surface plasmon resonance (SPR) of silver nanoparticles exhibits 10^5 to 10^6 times greater extinction cross sections than conventional molecular extinction cross sections. They also have excellent chemical and photostability, as well as being non-toxic to biological systems. Different plants have been accounted for, in the synthesis of metallic nanoparticles using plant extracts. There are, however, a huge number of plant metabolites that have yet to be investigated.

In our study we focussed on the photomediated green synthesis of silver nanoparticles from the aqueous rhizome extracts of *C. zanthorrhiza* Roxb. and *C. aromatica* Salisb. The phytochemicals of the rhizome extracts were qualitatively and quantitatively analyzed. The qualitative analysis revealed the presence of carbohydrates, free fatty acids and proteins, terpenoids, flavonoids, alkaloids, coumarins, phenols in CZ and CA rhizome extracts. The total phenolic content estimation using Folins'-Ciocalteau Reagent revealed significant amount of phenols. The HR-LCMS analysis of rhizome extract displayed the presence of eleven and thirteen bioactive compounds in the aqueous rhizome extracts of CZ and CA respectively. Harmanine was the most abundant compound in CZ and Ar turmenone was abundant in CA. These phytochemicals act as reducing and capping agents in the formation of silver nanoparticles.

The synthesized silver nanoparticles were characterized using the various techniques like UV-Visible Spectroscopy, FTIR, PXRD, HR-TEM, FESEM –EDAX, DLS and BET surface analysis. From the characterizations it was found that the synthesized silver nanoparticles were spherical, face centered cubic structure with size ranging from 10 nm to 50 nm. They were mesoporous with polycrystalline nature. From FTIR and HR LCMS analysis, the efficient capping of plant metabolites on to the synthesized silver nanoparticles was evident.

The catalytic property of silver nanoparticles was evaluated for the degradation of toxic dyes. The as synthesized silver nanoparticles effectively degraded the cationic (Malachite green) and anionic (Coomassie brilliant blue) dyes under sunlight and UV light illumination. The degradation of azo dyes (Orange G, Congo Red, Eriochrome Black T and Methyl Orange) were done using sodium borohydride as reductant and CZAgNPs and CAAgNps as catalyst. The degraded products were ascertained by HR-LCMS and found to be non-toxic. From the degradation studies the role of the AgNPs as effective nanocatalyst with promising activity and stability was identified. This highlights the use of CZAgNPS and CAAgNPs in environmental clean-up.

The antibacterial properties of the green synthesized silver nanoparticles were evaluated by disc diffusion and broth microdilution assay. It was revealed that the AgNPs were effective against both gram positive and gram negative bacterial isolates. The antifungal property of the nanoparticles against *A. niger* was studied by poisoned food technique and the results indicated that AgNPs inhibited the growth of the fungi in a dose dependent manner. Therefore the results proved the potential of the as synthesized silver nanoparticles as an efficient antimicrobial agent.

The antioxidant role of the synthesized silver nanoparticles was evaluated *in vitro* approach. The *in vitro* DPPH assay revealed the potential of CZAgNPs and CAAgNPS as radical scavengers inhibiting the activity of DPPH in a dose dependent manner. The short term *in vitro* activity of the as synthesized nanoparticles evaluated using trypan blue exclusion method in DLA and EAC cells exhibited remarkable cytotoxicity even at low doses validating that, the CZAgNPs and CAAgNPs can effectively inhibit the proliferation and growth of cancer cell lines. Further the green synthesized nanoparticles were subjected to MTT assay against the breast cancer cell lines (MCF – 7) and found to inhibit the growth and proliferation of MCF -7 cell lines. This inexplicably confirmed the ability of CZAgNPs and CAAgNPs as anticancer agents in cancer therapy.

In vivo toxicity assay is necessary to further evaluate the drugable property of green synthesized silver nanoparticles. Acute and sub-acute *in vivo* toxicity assay was conducted in Swiss albino male and female mice of weight 25 -30 g to evaluate the safety of the nanoparticles. The results showed no death and no significant change in the body weight and organ weight of animals administered with various doses of the silver nanoparticles. The biochemical and histological study did not show any significant aberrations. This illustrated that the synthesized silver nanoparticles were relatively non-toxic and safe for further applications at the administered doses (50 mg /kg b. wt, 75 mg /kg b. wt and 100 mg /kg b. wt.).

The protective role of the as synthesized silver nanoparticles evaluated *in vivo* by intoxicating sodium fluoride in mice models indicated the efficacy of the green synthesized silver nanoparticles to restore the antioxidant homeostasis.

The strong anticancer property of AgNPs was revealed by its higher efficacy in reducing the rapid growth and proliferation of DLA and EAC tumor in animal models when compared to the standard drug cyclophosphamide. The findings demonstrated that the green synthesized silver nanoparticles could cause toxic effects in DLA and EAC tumor models, halting tumor formation and successfully managing disease progression without damaging the normal cells.

The *in silico* molecular docking was studied using the tool AutoDock 4.2 which revealed the interaction between the selected protein molecules (bacterial, fungal, cancer cell surface) and the 13 ligands (the biomolecules bound on the as synthesized silver nanoparticles) based on the binding energy (ΔG) formed between the ligand molecules within the active site of the target protein. The results confirmed the potential of the biomolecules capped on the silver nanoparticles to inhibit gram positive, gram negative bacteria, fungi and cancer cell. This potentiality at the molecular level could be investigated for further analysis, especially in the area of drug designing.

Based on the findings, it was concluded that *C. zanthorrhiza* and *C. aromatica* rhizome extracts could be used to synthesize silver nanoparticles. This study also provides instrumental evidence for the biophysical properties of AgNPs generated from aqueous rhizome extracts of *C. zanthorrhiza* and *C. aromatica* by the different characterization methods. The biosynthesized AgNPs exhibited strong catalytic, antimicrobial, antioxidant, and anticancer properties, implying that they could be useful in environmental clean-up and pharmaceutical purposes. These findings add to the growing importance of biogenic AgNPs for prospective nanomedicine and nanocatalytic applications.

Future research directions

- *In situ* treatment of the polluted water bodies using the as synthesized nanocatalysts.
- Fabrication of microbe resistant clothing in hospitals and in wound dressing.
- The as synthesized silver nanoparticles could be used in food packaging as evident from antifungal studies.
- The antitumor activity generated by the green synthesized silver nanoparticles paves way for the development of potential and biologically safe antitumor drug after chronic toxicity studies and clinical trials.