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Original Article

Green Synthesis of Silver Nanoparticles from *Crossandra Infundibuliformis* Under Magnetic and Non-Magnetic Fields by Two Different Extraction Methods: Characterization and Bio Evaluation

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Abstract

Green synthesis of silver nanoparticles (AgNPs) offers a sustainable alternative to chemical methods, minimizing toxic by-products and enhancing biocompatibility. This study employed aqueous and ethanolic leaf extracts of Crossandra infundibuliformis under magnetic and non-magnetic conditions to evaluate the influence of solvent systems and magnetic field exposure on AgNP synthesis and activity. Nanoparticle formation was confirmed by colour change, FTIR with ethanolic extracts and magnetic exposure producing faster synthesis and stronger phytochemical involvement. Haemolysis assays indicated 85% red blood cell lysis, with ethanolic magnetic extracts showing higher activity, while magnetic treatment improved compatibility. Seed germination assays using Vigna radiata revealed that aqueous magnetic AgNPs significantly reduced root–shoot growth and biomass, whereas aqueous non-magnetic AgNPs had minimal phytotoxicity. Peroxidase is a stress-responsive enzyme. Changes in its activity in plant cells reflect nanoparticles induced oxidative stress. Biological assays highlighted antimicrobial potential as the most significant outcome. AgNPs demonstrated strong inhibition against Escherichia coli and Staphylococcus aureus, with ethanolic magnetic nanoparticles producing the largest inhibition zones. In contrast, aqueous non-magnetic nanoparticles showed comparatively weaker activity. These findings highlight that both solvent polarity and magnetic field exposure critically determine nanoparticle efficiency, with ethanolic magnetic AgNPs demonstrating superior antimicrobial potency but higher cytotoxicity. The work underscores the potential of C. infundibuliformis in eco-friendly nanotechnology and introduces magnetic field modulation as a novel strategy for tuning nanoparticle properties.

Keywords: *Silver nanoparticles (AgNPs), Crossandra infundibuliformis, Magnetic field exposure, Cytotoxicit*

Introduction

Nanotechnology is one of the most rapidly advancing scientific domains of the 21st century, playing a pivotal role in revolutionizing materials science, medicine, agriculture, and environmental engineering. Among the many nanomaterials studied to date, silver nanoparticles (AgNPs) occupy a distinguished position. Silver nanoparticles exhibit excellent antimicrobial, antifungal, antiviral, antioxidant, and cytotoxic properties, which make them valuable in biomedical devices, coatings, wound dressings, drug delivery systems, and agricultural disease management. In addition, AgNPs possess optical properties such as surface plasmon resonance, which enhance their use in sensing and diagnostic technologies. (Rai et al., 2009; Panacek et al.,2006). In this context, green synthesis has emerged as a revolutionary method of producing nanoparticles. Compared to microbes, plants are more advantageous because they are widely available, cost-effective, and rich in diverse phytochemicals that act both as reducing agents and stabilizers. Compounds like flavonoids, phenols, alkaloids, terpenoids, and reducing sugars present in plant extracts are capable of reducing silver ions (Ag^+) into silver nanoparticles while simultaneously capping them to prevent aggregation.

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This dual role ensures stability, uniformity, and biocompatibility of the synthesized nanoparticles. (Geoprincy et al.,2013; Kavitha et al.,2004) The present project employs *Crossandra* infundibuliformis, a flowering ornamental plant also known for its medicinal potential, as the bio-reducing agent. While this plant has traditionally been studied for its aesthetic and medicinal value, its role in nanotechnology remains relatively unexplored. Its phytochemical richness makes it an excellent candidate for nanoparticle synthesis. By utilizing both ethanolic and aqueous extracts of *Crossandra Infundibuliformis* leaves, this project captures the contribution of different classes of phytoconstituents toward nanoparticle formation. (Govindraju et al.,2010; Ahmed et al.,2011) An additional novel aspect of this study is the application of magnetic fields during the synthesis process (Geoprincy et al.,2013). Research has suggested that external factors such as light exposure, pH, and magnetic or electric fields can influence the kinetics of nanoparticle nucleation and growth. The presence of a magnetic field may alter the orientation of molecules, change ionic interactions, and affect the stability of colloidal systems. Although such influences remain underexplored, incorporating magnetic field exposure provides a new dimension to nanoparticle synthesis.

Methodology

1. Preparation of Plant Extracts

Collection and Processing of Plant Material:

Fresh leaves of *Crossandra infundibuliformis* were collected and identified by help of botany department Gogate Jogalekar college Ratnagiri, India. Leaves were thoroughly washed with distilled water to remove dust and surface contaminants, and shade dried at room temperature. The dried leaves were powdered using a clean grinder.

Aqueous Extract Preparation:

About 10 g of powdered leaves were boiled with 100 ml distilled water for 30 minutes, cooled, and filtered using Whatman No. 1 filter paper. The filtrate was stored at 4°C until further use.

Ethanolic Extract Preparation:

About 10 g of powdered leaves were soaked in 100 ml ethanol (95%) for 24–48 hours with occasional shaking. The solution was filtered and stored at 4°C until use.

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) solution was prepared freshly (1 mM). $AgNO_3$ solution and each extract (aqueous and ethanolic) were mixed in 3:1 ratio. For magnetic field synthesis, a simple bar magnet was placed beneath the reaction vessel throughout the synthesis period. Non-magnetic synthesis, the reaction was carried out without a magnet. All solutions were kept for 72 hrs in dark condition. Colour change of the solution (pale yellow to dark brown) was taken as preliminary evidence for nanoparticle formation. Synthesis was confirmed visually. Studies were done in four comparative Groups: (i)Aqueous extract – Magnetic field (AM), (ii)Aqueous extract – Non-magnetic (ANM), (iii)Ethanolic extract – Magnetic field (EM), (iv)Ethanolic extract – Non-magnetic (ENM). Particles were collected after centrifugation, giving numerous washings with distilled water. Nanoparticles were dried and dissolved in dimethyl sulfoxide solution (1mg in 10 µl). 100mg of particles dissolved in 100ml distilled water with help of sonication (Orchid scientific).

2. Fourier Transform Infrared Spectroscopy (FTIR) and TLC

Water samples were analysed by FTIR (Bruker alpha 11). A control spectrum of $AgNO_3$ solution was also obtained for comparison.

3. Haemolysis Assay

Fresh RBC suspension was prepared in saline. Different nanoparticle solutions (all four groups) were incubated with RBC suspension. (Saiqa A. et., 2020)

4. Phytotoxicity Assay

Seeds of *Vigna radiata* were grown in test solutions of nanoparticles (all four groups). Distilled water was used as control. Germination percentage, root and shoot length, cell biomass (fresh weight of germinated seedlings), protein content and peroxidase activity were studies (Bhushan B. et. Al. 2014) Seeds were grown on cotton bead soaked with water solution of 20 ml contains 20mg AgNPs concentration. Germination assay was done for 3 days.

Peroxidase Activity and Protein activity Assay:

Activity measured using guaiacol and hydrogen peroxide as substrates. Plant extracts treated with nanoparticles were incubated with guaiacol (0.5%v/v) H_2O_2 (30%). Formation of brown-coloured product indicated peroxidase activity. Colour intensity measured at 420 nm (Chance B. & Maehyl, A. C. (1955). Lowry method was used for protein content determination (Waterborg „J. H. (2009).

Vigna Radiata were grown in the sample solutions and distilled water was used as control. The comparison in grown of root and shoot was done. (Rajkuberan C. et.al., 2015), (Kim, et.al., 2011), (Andleeb S. et. Al., 2020)

5. Antimicrobial Assay: Antimicrobial assay was preformed per Jain and Mehata 2017. (Jain, S., & Mehata, S. 2017)

Results and discussion:

1. **Synthesis and Yield of Silver Nanoparticle:**

The formation of silver nanoparticles was evident by a gradual change in the solution colour from pale yellow to brown. Both aqueous and ethanolic extracts of *Crossandra infundibuliformis* facilitated this transformation, but the ethanolic extract showed a faster and more intense colour change, reflecting its higher content of reducing phytochemicals (Fig 1). Under magnetic field exposure, the colour development occurred more rapidly and produced a darker brown shade compared to the non-magnetic condition, suggesting an influence of magnetic fields on the kinetics and stability of nanoparticle formation. The obtained in yield in four group AM, ANM, EM, ENM was 54mg, 85mg,100mg,99mg respectively.

2. **FTIR Analysis:**

The biomolecules present in plant extract responsible for the reduction of silver ions to AgNPs were identified by FTIR analysis. FTIR spectra of AgNP show board O-H stretching (3300-3320cm^{-1}). Prominent aromatic bands are observed at 1600cm $^{-1}$ while carbonyl at 1700-1720cm $^{-1}$. Magnetic -condition syntheses induced shifts in several bands consistent with phytochemical silver interaction under magnetic conditions.

3. **Haemolysis Assay**

The haemolysis assay revealed that silver nanoparticles had a measurable effect on red blood cell (RBC) integrity (Fig3). The positive control (distilled water with RBC) resulted in complete haemolysis, while the negative control (saline with RBC) showed no haemolysis. All four nanoparticle groups induced partial haemolysis, though the degree varied. Nanoparticles synthesized with ethanolic extracts show higher haemolytic activity compared to aqueous extracts, reflecting stronger interactions with cell membranes. Interestingly, magnetic field-treated nanoparticles exhibited slightly higher haemolysis compared to their non-magnetic counterparts.

Phytotoxicity assay

Silver Nanoparticles can have both positive and negative effects on growth of germinating seeds, depending on their concentration, size, coating and exposure time. AgNPs can enter seed coats and root tissues altering water uptake and nutrient transport. Germination rate was 100% in all seeds.

Root, shoot growth halted after the treatment of all silver nanoparticles. (Table1) Root and also shoot growth was major affected by aqueous magnetic AgNPs. AM shows major reduction in growth as well as cell mass. AgNPs can enter seed coats and root tissues altering water uptake and nutrient transport.

Peroxidase Activity and protein content

Peroxidase is one of the major stress enzymes. Increase in the activity observed in EM And ENM while decrease in activity observed in AM and ANM. AM extract also shows 80% reduction in cell mass. Decrease in peroxidase activity is due to less protein content.

Antimicrobial Assay

It is well known that AgNPs have a higher surface to volume ratio as compared to their bulk counterpart. Therefore, some interactions with the bacterial surfaces are facilitated, and antibacterial property of AgNPs is enhanced. Reports show that the interaction of AgNPs with the sulphur and phosphorus containing constituents of the bacterial cell initiates cell killing by attacking the respiratory chain and cell division (Rai et al., 2009). Also, it was seen from disk diffusion method (Table.3 and Fig.4) that AgNPs synthesized using all four-extraction method had antibacterial activity, but gram-positive growth more inhibited compare to gram negative bacteria. Both aqueous as well as ethanolic magnetic AgNPs shows more inhibition compare to nonmagnetic.

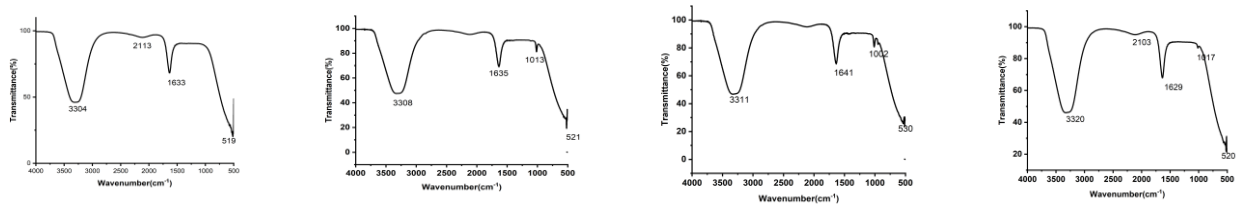
Observations:

Synthesis of Silver Nanoparticles:

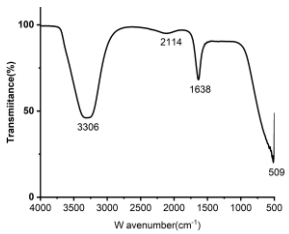
(a) (b) (c) (d)

Fig 1: Synthesis of nanoparticles (a) EM (b) ENM (c)AM (d)ANM

FTIR Characterization:



Sample 1 Sample 2. Sample 3 Sample 4



Sample 5

Fig 2: Characterization of silver synthesised silver nanoparticles
(sample 1) ANM, (sample 2) ENM, (sample 3) AM, (sample 4) EM, (sample 5) Control.

Haemolysis:

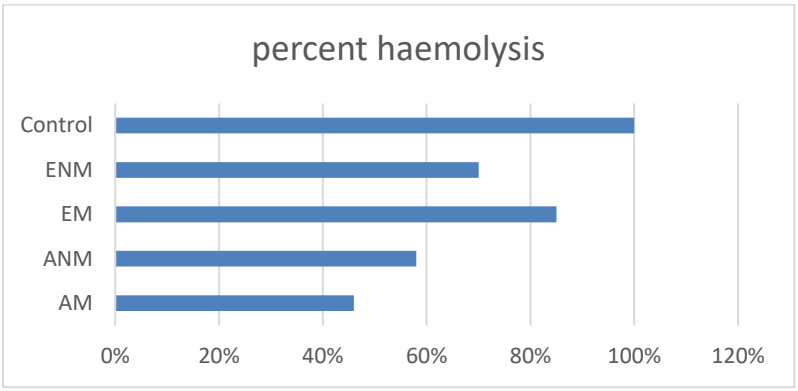


Fig 3: Percent haemolysis

Phytotoxicity Assay:

Root Shoot Ratio and Cell Biomass

Table 1: Statistical Analysis of Root Shoot Assay

SAMPLE	ROOT		SHOOT		CELL BIOMASS
	MEAN	RSD	MEAN	RSD	
AM	0.9 ± 0.09	10	1.09 ± 0.14	12.84	2.380
ANM	2.93 ± 0.55	18.77	3.14 ±0.42	13.3	3.072
EM	4.2 ± 0.40	9.52	4.31 ±0.25	5.8	3.396
ENM	2.59 ±0.48	18.53	3.01 ± 0.61	20.2	2.250
DISTILLED WATER	4.4±0.38	8.63	8.58 ± 0.48	5.59	4.240

Peroxidase Activity and Protein Estimation

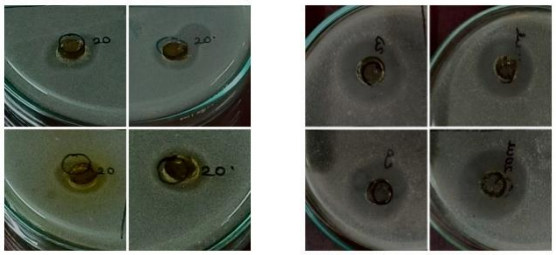
Table 2: Peroxidase activity and Enzyme Estimation

Sample	Peroxidase activity	Enzyme protein
AM	16.91	0.9
ANM	10.15	2.7
EM	22.55	1.8
ENM	21.80	2.7
DISTILLED WATER	19.92	4.5

Antimicrobial Activity:

Table 3: Inhibition zones by agar well diffusion

Sample Name	E. coli		S. Aureus	
	0.4 mg	0.8 mg	0.4 mg	0.8 mg
AM	1.3	1.5	2.1	2.3
ANM	1.4	1.6	2.3	2.5
EM	1.6	1.7	2.2	2.4
ENM	1.4	1.5	2.3	2.5



(a) (b)

Fig 4: Zone of Inhibition observed by Agar well Diffusion.
(a) E. coli (b) S. aureus.

Conclusion

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using aqueous and ethanolic extracts of Crossandra infundibuliformis leaves, under Both magnetic and non-magnetic field conditions. This comparative approach allowed the Assessment of how extract type and external magnetic exposure influence nanoparticle Synthesis and their subsequent biological properties. The initial confirmation of nanoparticle Formation was evident through a visual colour change, followed by characterization using TLC And FTIR, which confirmed the involvement of phytochemicals and the successful reduction of silver ions into nanoparticles.

Comparative studies showed that ethanolic extract generally exhibited higher activity than Aqueous extract, and the application of magnetic field showed subtle but distinct variation in Nanoparticles, as reflected in assays and phytochemical mobility. Biological assays, including haemolysis, cytotoxicity, root-shoot ratio, peroxidase activity, Protein estimation, and antimicrobial activity, indicated that the synthesized nanoparticles Possess significant bioactive potential. While haemolysis and cytotoxicity highlighted Concentration-dependent cellular effects, the antimicrobial assays confirmed notable inhibition Against E. coli and S. aureus. Overall, this project establishes Crossandra infundibuliformis as a promising plant source for Eco-friendly nanoparticle synthesis, with both material and biological relevance. The observed Influence of magnetic field treatment opens new perspectives in tailoring nanoparticle Properties for biomedical, agricultural, and environmental applications.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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