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In vitro Anticancerous and Antimicrobial Activities of *Curcuma amada* Mediated Silver Nanoparticles

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ABSTRACT: Silver nanoparticles are extensively studied due to their exceptional physicochemical and biological features. Phytocompound based silver nanoparticles are considered as a unique and potentially useful alternative process for chemically synthesized nanoparticle. *Curcuma amada* rhizome mediated Ag NPs anticancerous and antimicrobial activities were analyzed. *In vitro* anticancerous studies against human breast cancer (MDA MB-231) cell lines and antimicrobial studies against gram positive (*Staph. aureus, Bacillus subtilis*) and negative (*Pseudomonas aeruginosa*, *Escherichia coli*) were studied. Increased concentrations of the silver nanoparticles showed increased cytotoxicity in the tumor cell lines (MDA MB-231) with IC50 observed as 163.5µg/ml. Among the tested bacterial species with different concentrations (10, 20, 40, 60, 80, 100, 200, 300, 400 and 500µg/ml) of green Ag NPs, *P. aeruginosa* showed high growth inhibition (14.7±0.8mm.) at 30µg/ml. Our study concluded that green synthesized Ag NPs by *C. amada* rhizome extract showed an efficient anticancerous and antimicrobial properties.

Keywords: Silver Nanoparticles, *C. amada*, Anticancerous, Inhibition Zone, Cytotoxicity.

INTRODUCTION

In the field of nanotechnology, silver nanoparticles (Ag NPs) stand out among the noble metals because of their exceptional qualities, including chemical inertness, good conductivity, catalytic activity, and, most importantly, their antibacterial, antiviral, antifungal, and anti-inflammatory nature (Aisida *et al.*, 2021; Lekshmi *et al.*, 2022). These qualities allow them to be used in composite fibres, cosmetics, supercooled semiconducting materials, food and electron beam industries (Klaus-Joerger *et al.*, 2001; Ahmad *et al.*, 2003). In biomedical uses, silver used in antiseptic creams and has a wide toxic effect on bacteria by rupturing cell membrane and altered their enzymatic mechanism. Also used to treat wound and acne, antiseptic sprays, textiles and ointments (Palanisamy *et al.*, 2017). For the synthesis of silver NPs, a range of procurement approaches have been used, with plant extracts being the most appealing due to the accessibility of eco-friendly procedures, biological entities, abundance, cost-effectiveness, safety, and regenerated for human medicinal use (Kumar *et al.,* 2009).

Plant-based green synthesis is cost-effective, sustainable, quick, and biodegradable (Singh *et al.*, 2018) due to their phytoconstituents with variety of pharmacological effects (Kathirvel *et al.,* 2016). One approach that is advantageous compared to other ones that are accessible is the green production of nanoparticles (Das *et al.* 2017). Green synthesis played a crucial role in producing metal oxide NPs because of simple and effectiveness production mechanism in the nanomedicine field (Devanesan *et al.,* 2021). Several studies have shown that phytochemical substances such as lignins, flavones and terpenes can activate the antimicrobial and antitumor properties of phyto-synthesized AgNPs (Gopinath *et al.*, 2016). In plants, due to the reducing characteristics dissociation of metal compounds in their respective nanoparticles is commonly observed whereas microorganisms such as bacteria, fungus, and plants are utilized in biological processes to green synthesis of silver nanoparticles (Hailan *et al.*, 2022). Loo *et al.* (2012) reported that phytocompound mediated Ag NPs production considered as a more favorable process than other biological methods since it doesn't need any maintenance of culture cells and disease free conditions. The main objectives of our study is to analyze the impact of anticancerous and antimicrobial activities of the *C. amada* mediated Ag NPs.

MATERIAL AND METHODS

Curcuma amada rhizome mediated silver nanoparticles synthesis process and nanoparticle characterization studies were confirmed the presence of nanosize in the synthesized particles (results were not included here). Different concentrations of the *C. amada* mediated Ag NPs were prepared and utilized for the anticancerous and antimicrobial studies.

A. Anticancerous studies

The human breast cancer (MDA MB-231) cell lines were cultured in 5% CO_2 at 37°C. The cells were mycoplasma-free and grown in fresh media (RPMI1640) nourished with 10% FBS and 1% penicillin or streptomycin. The cells (10^6cells/ml) were seeded in T-25 beakers containing 5 ml RPMI1640 and grown to sub-confluence (90-95%) in a humidified incubator (95% air; 5% CO_2) at 37°C (Bray *et al*. 2018). Every 48 hours, the culture media was changed. To achieve full cell dissociation, the single layered cells were treated with 1ml (0.25%) EDTA and quickly incubated. The cells were resuspended in full growth media. Trypan (0.4%) blue stain was used for staining and counted using a hemacytometer, and incubated for 2 min (Noorbazargan *et al.*, 2021). Before treatment, the cells were plated (10^4 cells well^{-1}) in 96 wells microtiter plates.

B. Antimicrobial studies

C. amada mediated Ag NPs antimicrobial properties against gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative (*Pseudomonas aeruginosa*, and *Escherichia coli*) bacteria were tested by using disc diffusion method (Guzman *et al.*, 2012). On the Petri dishes with the autoclaved Mueller Hinton Agar (MHA) medium various bacterial strains were dispersed. On separate Petri dishes filled with LB media, the discs (6mm) dipped with known concentrations of standard and green synthesized Ag NPs were then inoculated. The cultures ware incubated at 37°C and the inhibition zone for each disc were observed after 18 hours by using a ruler.

RESULTS AND DISCUSSION

Different concentrations of *C. amada* rhizome mediated Ag NPs as 10, 20, 40, 60, 80, 100, 200, 300, 400 and 500µg/ml were tested against MDA MB-231 human breast cancer cell lines (Fig. 1). Increased concentrations of the silver nanoparticles showed increased cytotoxicity in the tumor cell lines (Fig. 2). The median inhibitory concentration (IC50) of the MDA MB-231 cancer cell lines were observed as 163.5µg/ml. Figure 2 showed the reduced number of cells in the culture media as increased concentrations of the green synthesized Ag NPs.

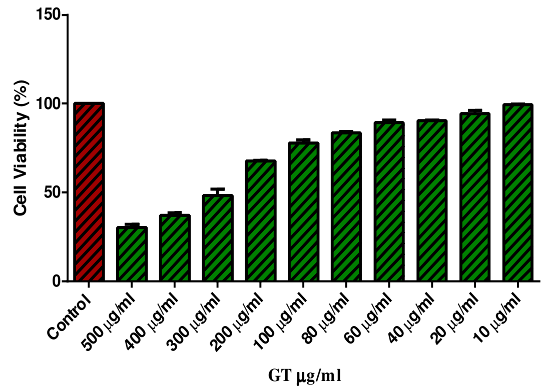

Fig. 1. Cytotoxicity effect of *C. amada* mediated Ag NPs on MDA MB-231 human breast cancer cell line.

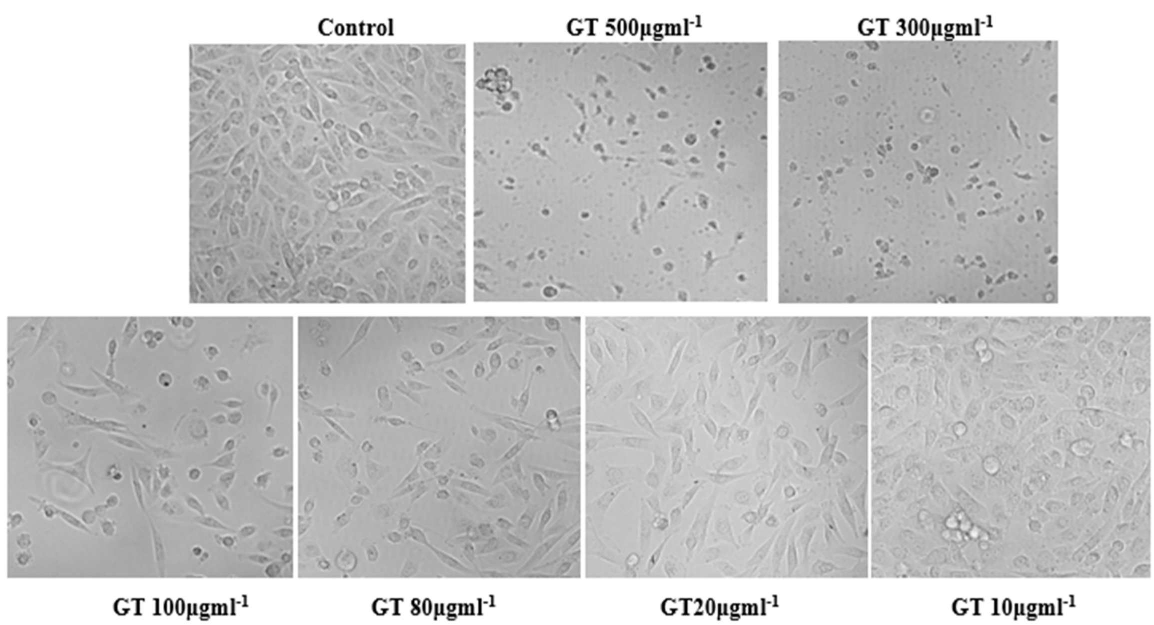

Fig. 2. Effect of *C. amada* mediated Ag NPs on human breast cancer cell lines.

Table 1: Antimicrobial activity of *C. amada* mediated Ag NPs against selected microbes.

Microorganisms	Zone of inhibition (mm)				
	$10 \mu g ml^{-1}$	$15 \mu g ml^{-1}$	$20 \mu g ml^{-1}$	$25 \mu g ml^{-1}$	$30 \mu g ml^{-1}$
Staphylococcus aureus	10.1±0.5	11.2±0.3	11.9±0.7	12.4±0.9	13.5±0.6
Bacillus subtilis	10.7±0.4	11.6±0.6	12.5±0.7	13.2±0.6	14.1±0.8
Pseudomonas aeruginosa	11.7±0.8	12.6±0.7	13.3±0.5	13.9±0.7	14.7±0.8
Escherichia coli	9.5±0.4	10.1±0.9	10.8±0.6	11.5±0.6	12.4±0.8

In vitro anticancerous studies on Hepatocellular carcinoma cells (Hep G2) lines showed a significant inhibition of tumor cell growth by Ag_2ONPs, enhanced by autophagy, reduced mitochondrial membrane osmolality and high DNA damage (Sujatha *et al.*, 2023). Mir *et al.* (2022) reported the anticancerous activity of the silver nanoparticles especially on the increased ablation activity on the cancer cell lines. *Lonicera hypoglauca* flower extract mediated AgNPs has potent anticancer properties and functions as capping and reducing agents in human breast cell lines (MCF-7). AgNPs synthesized from the pulp extract of *Abelmoschus esculentus* shown anticancer and antibacterial activities. The cytotoxicity concentration (IC50) of CfAgNPs was 50 g/ml. The viability of MCF-7 cells declined when CfAgNP concentrations rose (Ulagesan *et al.*, 2021). The effectiveness of biologically produced AgNPs-GA as a cytotoxic agent against MCF - 7/TAMR – 1 and MCF - 7 human breast cancer cell lines. Both the human breast cell lines are significantly inhibited from proliferating by AgNPs-GA, with IC50 at 34.0 and 2.0g/mL respectively, after 3days of exposure (Zulkifli *et al.,* 2020).

Different concentrations (10, 15, 20, 25 and 30µg/ml) of *C. amada* mediated Ag NPs has growth inhibition activity on gram positive and gram negative bacterial species. Increased concentrations of the nanoparticles significantly increased the growth inhibition of the treated bacterial species. The inhibition zone (10µg/ml) were observed as 9.5±0.4, 10.1±0.5, 10.7±0.4 and 11.7±0.8mm for *E. coli, Staphy. aureus, B. subtilis* and *P. aeruginosa* respectively. In 15µg/ml group, the inhibition zones were observed as 10.1±0.9, 11.2±0.3, 11.6±0.6 and 12.6±0.7mm for *E. coli, Staphy. aureus, B. subtilis* and *P. aeruginosa* respectively.

Similar increased inhibitory results were observed in 20µg/ml and 25µg/ml concentrations as 10.8±0.6, 11.9±0.7, 12.5±0.7, 13.3±0.5mm and 11.5±0.6, 12.4±0.9, 13.2±0.6, 13.9±0.7mm respectively for *E. coli, Staphy. aureus, B. subtilis* and *P. aeruginosa.* In 30µg/ml group among the tested bacterial species, *P. aeruginosa* showed high growth inhibition as 14.7±0.8mm whereas the least growth inhibition was observed against *E. coli* as 12.4±0.8mm (Table 1). Phytocompounds present in the synthesized nanoparticles altered the bacterial membrane integrity by producing ROS which leads to the bacterial cytotoxicity. Antimicrobial effect of *C. amada* rhizome extract mediated Ag NPs against gram negative and positive bacteria *P. aeruginosa>B. subtilis>Staphy. aureus>E. coli.*

According to Haes *et al.* (2002) Ag NPs using a green technology have uses in both biomedicine and the management of harmful microorganisms. Changes in cell shape and viability, metabolism, and oxidative stress were used to assess the toxicity. Phytocompound (*P. longum*) mediated Ag NPs enhanced the amount of ROS generated by the cell and damaged the mitochondria in a dose-dependent way (Reddy *et al.,* 2014). At a concentration of 40g/mL, the *D. montana* bark mediated Ag_2O NPs showed a substantial zone of growth inhibition in both gram-negative and positive bacteria (*E. coli*-16.3±2.6mm, *P. aeruginosa*-8.6±1.6mm and *B. subtilis*-22.3±4.5mm, *Staph. aureus*-18.7±3.1mm) (Sujatha *et al.*, 2023) which supported our results. Phytocompounds present in *C. amada* rhizome involved a chelation reaction with the Ag NPs showed an effective growth inhibition against the multi drug resistant variant *P. aeruginosa.*

In the present study, *C amada* rhizome mediated silver nanoparticles exhibited antimicrobial effect on *P. aeruginosa, E. coli, Staph. aureus* and *B. subtilis*. This is due to the significant potent of these silver nanoparticle on ROS production in microbial cytoplasm which lead to cell lysis (Yin *et al.,* 2020). Green synthesized Ag_2O NPs demonstrated greater zone of inhibition and maximal antibacterial effectiveness against gram positive than gram-negative bacteria. Phytomediated Ag_2ONPs exhibit excellent efficacy as an efficient supply of medications that quickly lessen bacterial-oriented disorders (Mollick *et al.* 2019; Fayyadh *et al.,* 2021).

CONCLUSIONS

Green chemistry of Ag NPs using *C. amada* rhizome is a concise, safe and one-step procedure. Phytocompounds present in the synthesized nanoparticles elevated the reduction reaction on the tumor cells and pathogenic microorganisms. Altered membrane integrity resulted in the cytotoxicity of the tumor cells and the microbes.

FUTURE SCOPE

To produce *C. amada* based green metallic nanoparticles which showed an effective role in the industrial and remediation sectors and also helps to reduce the toxicity developed by the chemical based nanoparticles.

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Conflict of interest. None.

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