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Original article

Green synthesis, characterization, enhanced functionality and biological evaluation of silver nanoparticles based on *Coriander sativum*

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ABSTRACT

The present study focused on the green synthesis of silver nanoparticles from *Coriander sativum* (CS) containing structural polymers, phenolic compounds and glycosidic bioactive macromolecules. Plant phenolic compounds can act as antioxidants, lignin, and attractants like flavonoids and carotenoids. Henceforth, silver nanoparticles (AgNPs) were prepared extracellularly by the combinatorial action of stabilizing and reduction of the CS leaf extract. The biologically synthesized CS-AgNPs were studied by UV-spectroscopy, zeta potential determination, scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis to characterize and confirm the formation of crystalline nanoparticles. The synthesized nanoparticles demonstrated strong antimicrobial activity against all microbial strains examined with varying degrees. The scavenging action on free radicals by CS-AgNPs showed strong antioxidant efficiency with superoxide and hydroxyl radicals at different concentrations as compared with standard ascorbic acid. The presence of *in vitro* anticancer effect was confirmed at different concentrations on the MCF-7 cell line as revealed with decrease in cell viability which was proportionately related to the concentration of CS-AgNPs illustrating the toxigenic nature of synthesized nanoparticles on cancerous cells.

1. Introduction

Silver nanoparticles applications are widespread from food processing, cosmetics, home cleaning, garment manufacturing to medicinal applications (Bansod et al., 2015; Benn et al., 2010; Tulve et al., 2015). Silver nanoparticles can be synthesized via various paths, including electrochemical, nuclear, photochemical and biological methods (Lombardo et al., 2016; Malkar et al., 2014; Treshchalov et al., 2017). As chemical synthesis can lead to undesirable environmental toxic effects, phytochemical synthesis using biopolymer (Pandey et al., 2012), chitosan (Prabaharan, 2015), cellullose (Abdollahi et al.,2013), gum Arabic (Kong et al., 2014), plant extracts (Dakshayani et al., 2019; Nazar et al., 2018) and essential oils (Esmaeili and Asgari, 2015) is an eco-friendly process for synthesizing nanoparticles. A variety of plant extracts that are capable of ion reduction, and at low cost of production are employed for the production of nanoparticles. (Combo et al., 2013; Das and Brar, 2013). *Coriander sativum*, CS is a common spice and a major curry powder ingredient possessing antimicrobial, hypolipidemic, hypoglycemic action and insecticidal effect (Hwang et al., 2014; Mandal and Mandal, 2015). This is one of North Africa, Southern Europe, and Southwest Asia's commonly cultivated herbs. A number of macromolecules are primarily responsible for its biological activity. Presence of aldehyde compounds is largely responsible for the aroma of coriander leaves. The aldedydes with 6–10 carbon atoms constitutes major proportion of coriander leaves. Plant phenolic compounds (lignins and flavanoids) can serve as antioxidants, signaling compounds and as chemicals for the defense response like tannins. Furthermore, protective properties as anti-ageing, antioxidant, anti- proliferative and anti-inflammatory owes to the occurrence of phenolic compounds. Coriander is used for digestive issues including stomach discomfort, appetite loss, hernia, nausea, vomiting, bowel spasms and bowel gas. It is also used to

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treat measles, hemorrhoids, toothaches, worms and joint pain, as well as bacterial and fungal infections (Cortes-Eslava et al., 2004; Rattanachaikunsopon and Phumkhachorn, 2010; Samojlik et al., 2010; Sharma et al., 2010; Silva et al.,2011).

When plant extracts are utilized in production of silver nanoparticles via reduction and stabilization of silver nanoparticles, they do not bear chemical compounds on their surface and hence not harmful to human cells (David and Moldovan, 2020). The scavenging effect of Ag-NPs against free radicals owes to the phytochemicals adhering on the surface of nanoparticles (Ansar et al., 2017; Ansar et al., 2018). Critical aspects including the option of the plant to use established plant potential, the antioxidant, antimicrobial, anti-inflammatory and antimicrobial activities from various regions of the earth need to be considered (Vijayan et al., 2018; Vijilvani et al.,2020; Wang et al., 2020a; Wang et al., 2020b). In comparison to the chemical based synthesis, the green synthesized silver nanoparticles exhibit reduced cytotoxicity and henceforth applicable in biological purposes, as in treatment of infectious diseases which are contagious and particularly employing topical therapies (Vijayan et al., 2018; Wu et al., 2020; Zorraquin-Pena et al., 2020).

Numerous biological properties of bio-based silver nanoparticles are well documented including antioxidant, antimicrobial, anticancer and tissue healing (David and Moldovan, 2020; Ansar et al., 2017; Ansar et al., 2018; Vijayan et al., 2018) Furthermore, there are various applications of Ag-doped semiconductor nanoparticles in enhancement of photo-conversion yield; in widening of light absorption of semiconductors to visible light; and in photocatalytic reactions viz. organic pollutant degradation, production of hydrogen, disinfection and photoreduction of CO_2 (Vijilvani et al., 2020). The major concern with respect to human health is the development of antimicrobial resistance observed in recent periods with devastating effect on mankind and economics. Metals like gold, aluminium, iron oxides and silver has been studied to possess antimicrobial applications (Ahmed et al., 2016). These "nanoantibiotics" are more advantageous than the traditional agents due to decreased susceptibility to bacterial resistance. Furthermore, cancer is the most prevalent disease (second leading cause of death in men) characterized by uncontrolled cell division. The metastasis in asymptomatic cases makes the diagnostic and therapeutic fields more challenging. The anticancer drugs not only affect tumourous cells but unfortunately targets the normal cells too (Gallo et al.,1993). Henceforth, finding a nontoxic lead from natural sources has become more important. In this study, synthesis of biogenic silver nanoparticles bringing the advantages of *C. sativum* for its antimicrobial, antioxidant and anticancer applicability has been accomplished.

2. Material and methods

A Schematic representation for the synthesis and evaluation of biological activity of CS-AgNP is depicted in Fig. 1.

2.1. Synthesis of CS-AgNPs

30 gm of *C. sativum* leaves (fresh) were gathered from a local market. The leaves were thoroughly rinsed with distilled water and boiled for 20 min in 250 ml of ultrapure water, sieved and stored. Later the extracts were subjected to filtration for synthesis of CS-AgNPs. For preparation of CS-AgNPs, Coriander extract was added to $AgNO_3$ aqueous solution 0.001 M in 1:10 dilution at room temperature and incubated for 10 min for reduction of silver ions (Yu et al., 2019). This resulted in brownish yellow solution, thus confirming the formation of Ag-NPs. Suitable control was set up along with experiment.

2.2. Characterization by UV–Vis spectrophotometer

Aqueous solution of Ag-NPs was scanned using UV–Vis spectrophotometer to obtain the absorption maxima from 300 nm to 600 nm. Hitachi S-4500 SEM machine was used to capture Ag-NPs. Samples were prepared by applying synthesized silver nanoparticles drop wise on a grid layered with copper and dried for 10 min under mercury lamp. Zeta potential analyzer ZEN3600, Malvern was used for hydrodynamic size and zeta potential analysis of the Ag-NPs. The analyzer principally involves irradiating the particles in suspension of medium viscosity 0.887 mPas with red laser beam 633 nm at 173° scattering angle at a temperature of 25 °C. The presence of silver and other elements

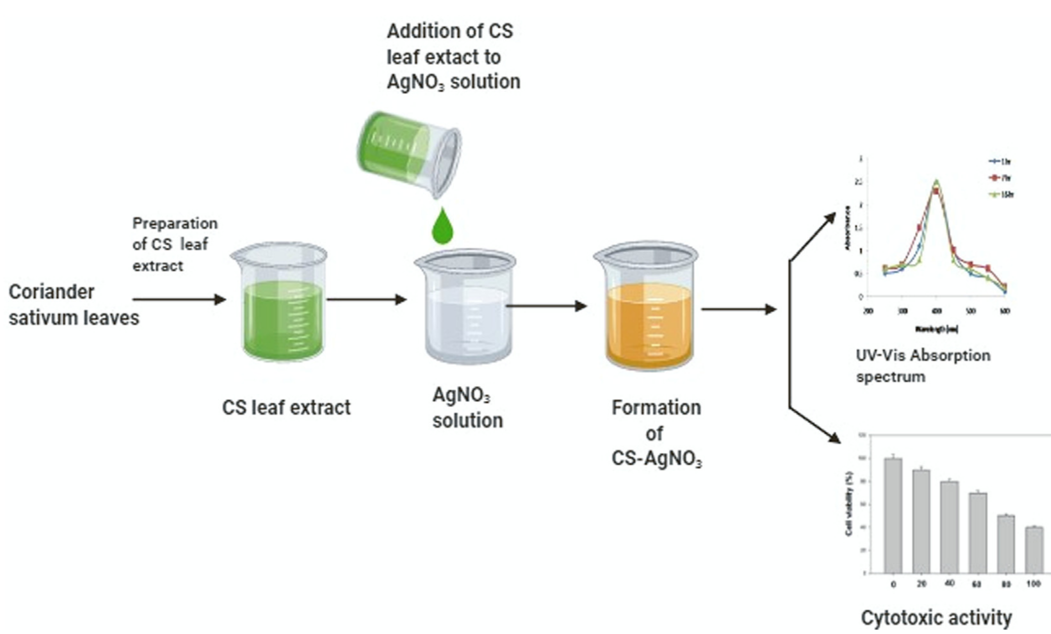

Fig. 1. Schematic representation for the synthesis and evaluation of biological activity of CS-AgNP.

in particles was confirmed by EDX analysis using high-resolution Scanning electron microscope JEOL JEM 2100.

2.3. Estimation of antibacterial activity

Ag-NPs produced were tested for inhibitory activity against varied bacterial strains. For testing antibacterial activity, 100 µl inoculum (about 10^8 CFU/ml) of each bacterial strain was mixed with Mueller Hinton agar (18 ml), poured in 90 mm petri dishes and allowed to settle. The discs were saturated separately with double distilled water, silver nitrate as positive control and Ag-NP solution and later dried under aseptic conditions. The concentration of AgNP/disc was 25 µg/mL. The inhibition zones were visualized after incubating samples for 16 h at 37 °C and sample demonstrating highest zone of inhibition was recorded.

2.4. Cytotoxicity studies

Hormone-dependent human breast cancer cells-MCF-7 were used in this assay. The medium used for the growth of the cell lines was the Eagle minimum essential medium (EMEM) supplemented with fetal bovine serum FBS-10% with suitable conditions of growth −37 °C and 5% CO_2. A final cell density of 1×10^5 cells/ml was obtained by diluting with medium containing 5% FBS. For cell attachment, 96 well flat bottomed plate were seeded with MCF-7 cells in their exponential phase of growth at 37 °C with 5% $CO_{2,}$ 100% relative humidity, and 95% air. Increasing concentration of CS-AgNPs from 0 to 100 µgm/ml was loaded and kept for 48 h under incubation. Following incubation, MTT reagent; 3-(4,5 -Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/mL) prepared in PBS was added. The medium served as control. The plates were incubated further at temperature of 37 °C for 180 min. Determination of cell viability was performed after reading plate at A_{570} nm.

2.5. Determination of antioxidant activity

2.5.1. Superoxide anion radical-scavenging assay

Varied concentrations of CS-AgNPs (50–200 µg/ml) and approximately 1 ml of the reaction mixture comprising of phosphate buffer (100 mM, pH 7.4), NADH (468 µM), NBT (156 µM), and PMS (60 µM) were mixed at the ambient temperature and incubated for 5 min. The formation of purple formazan (nitroblue tetrazolium) marks the superoxide anion radical-scavenging assay detectable at 560 nm in a spectrophotometer. Nicotinamide adenine dinucleotide contribute to generation of superoxide radicals which are detoxified by the prepared CS-AgNPs (Nishikimi et al., 1972)

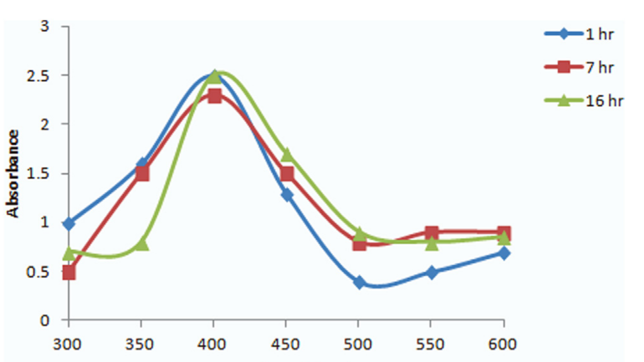

Fig. 2. UV–Vis spectra showing absorbance with silver nanoparticles from CS leaf extract with different time intervals.

2.5.2. Hydroxyl radical-scavenging assay

Hydroxyl radical scavenging assay was performed preparing a reaction mixture of 3 ml containing salicylic acid and ferrous sulphate, 9 mM each and mixed with 1 ml of hydrogen peroxide. One ml of prepared CS-AgNps was added to the above mixture at varying concentrations, mixed and incubated for 60 min at 37 °C. Post incubation, the absorbance values at A_{510} nm were recorded. A negative control was run parallelly and the percentage (%) of hydroxyl radical-scavenging activity for the test samples was then determined (Smirnoff and Cumbes, 1989).

2.6. Statistical analysis

Statistical analysis was performed using SPSS 17.0 software. Level of significance was evaluated by running One-way analysis of variance ANOVA. Each experiment was performed in triplicates n = 3 and mean values were reported.

3. Results

3.1. Ulraviolet-violet spectroscopy

The formation of green AgNPs synthesis was demonstrated by visual color change (light green to dark brown) after completion of reaction between CS extract and silver nitrate. The resultant solution exhibited a constant λ_{max} at 400 nm confirming the synthesized AgNPs' regulated size and shape as in Fig. 2 at varied intervals of time.

3.2. Results of scanning electron microscopy SEM

SEM image of the high-density green AgNPs synthesized further confirmed the development of silver nanostructures Fig. 3. The SEM micrographs of the NPs obtained in the filtrate showed that AgNPs were spherical shaped and well distributed in the solution without aggregation.

3.3. Results of dynamic light scattering spectroscopy

An additional function of nanoparticles that quantifies charge is the ZP viz Zeta potential. It is an index of nanoparticle's active electrical charge on its surface. The ZP provided details on the stability of the particles. The greater the potential, the greater the repulsion and stability of the electrostatics. The distribution of zeta potential graph is shown in Fig. 4.

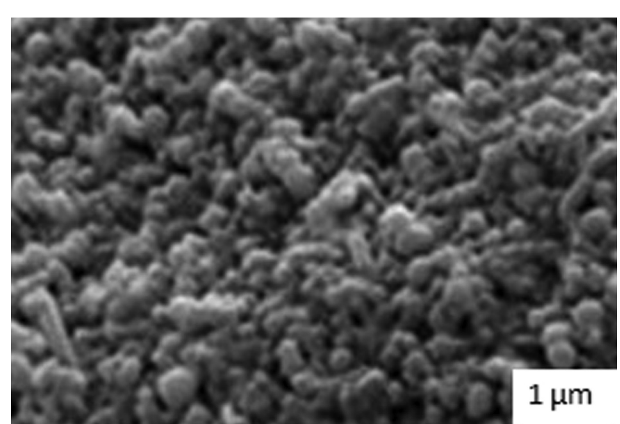

Fig. 3. SEM micrograph of the AgNPs prepared with aqueous CS aqueous leaf extract.

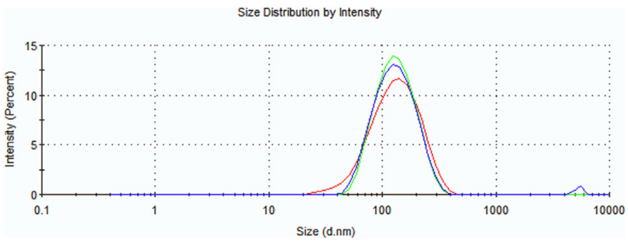

Fig. 4. Average size of Nano silver prepared with aqueous CS leaf extract.

3.4. Energy dispersive X-ray EDX analysis

The EDX profile showed a good silver signal together with remarkably stronger peaks confirming that the pellets were AgNPs. The elements that bind on Ag-NPs surface are thought to be originated from phytochemicals present in the plant extract and are depicted in Fig. 5 along with typical silver peaks, thus confirming the formation of CS-AgNPs using CS extract.

3.5. Cytotoxicity assay

The cytotoxic effects of CS-AgNPs was evidenced by MTT assay after effectively reducing the MTT dye. The cell viability was found to be inversely dependent on the dosage of biosynthesized silver nanoparticles. Results demonstrated that the cytotoxicity was exhibited on MCF-7 by the plant extract capping the nanoparticles. Antagonistic effect of CS-capped Ag-NPs on MCF-7 cells demonstrated cell death at higher concentrations with a IC_{50} value of 45 µg/ml, (Fig. 6). The cell viability was determined from the percentages of viable cells and untreated controls.

3.6. Antimicrobial assay

Green synthesized Ag-NPs were tested for antimicrobial activities against varied bacterial strains by the zone of inhibition study. Table 1 depicts the Disc diffusion assay expressed as zone of inhibition ZI. The zone of inhibition was in the range of 11–13 mm and 9 to 10 mm in diameter against Gram-positive and Gram negative bacteria respectively. Based on the above results, the antibacterial activity of the biosynthesized Ag nanoparticles exhibited was more pronounced against Gram positive bacteria.

3.7. Antioxidant assay

The superoxide and hydroxyl radical scavenging action of the green synthesized CS-AgNPs are shown in Table 2 and 3 with ascorbic acid as standard antioxidant. The percentage of radical scavenging abilities increased with increase in concentrations of CS-AgNPs (50–200 µg/ml) thus demonstrating its antioxidant property. Around 43–74% and 39–72% of the superoxide radical and hydroxyl scavenging activity of CS-AgNPs respectively with maximum activity at 200 µg/ml were observed.

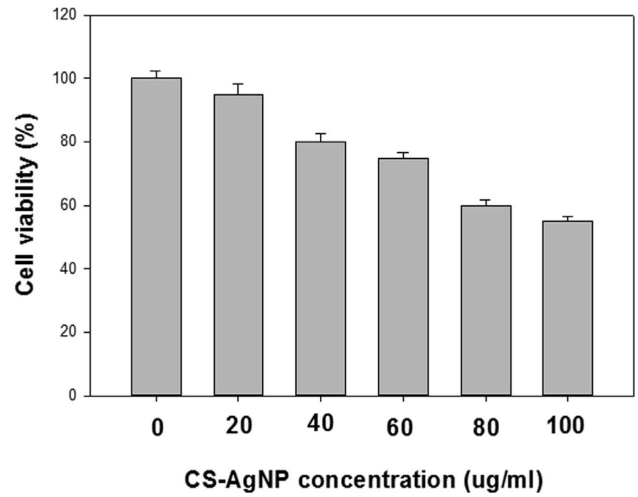

Fig. 6. Antagonistic action of CS-AgNPs on MCF-7 cells

Table 1
Antibacterial activity of CS-AgNPs aqueous leaf extract against bacterial species tested using disc diffusion assay.

Bacterial strains	Zone of Inhibition(mm)	
	Control	CS-AgNPs
Bacteroides fragilis (ATCC 25285)	10	11
Staphylococcus epidermidis (ATCC 12228)	9	12
Staphylococcus aureus (ATCC 6538)	12	13
Enterococcus faecalis (ATCC 33186)	10	11
Streptococcus pneumoniae (ATCC 10015)	11	12
Proteus mirabilis (ATCC 12453)	9.5	10
Klebsiella pneumoniae (ATCC 10031)	8	9
Escherichia coli (ATCC 25922)	8.5	10
Pseudomonas aeruginosa (ATCC 9027)	7	9

4. Discussion

Recent years have shown wide applications of Ag-NPs which made researchers to concentrate on developing novel synthetic

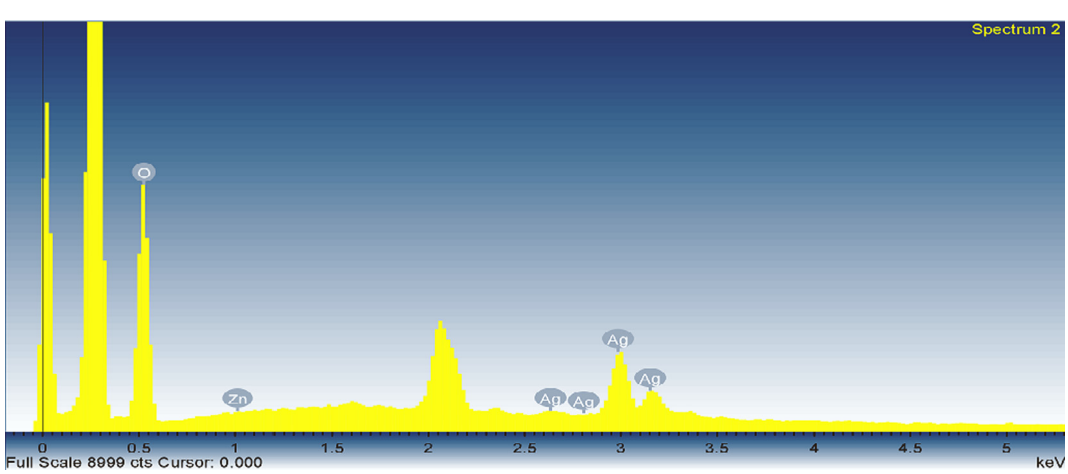

Fig. 5. EDX elemental analysis of the AgNPs prepared with aqueous CS aqueous leaf extract.

Table 2
Superoxide radical scavenging ability of CS-AgNPs.

Concentration of synthesized nanoparticles (μg/ml)	Scavenging activity for synthesized nanoparticles (%)	Scavenging activity for Ascorbic acid (%)
50	43	78
100	52	88
150	64	92
200	74	95

Table 3
Hydroxyl-scavenging activity of CS-AgNPs.

Concentration of synthesized nanoparticles (μg/ml)	Scavenging activity for synthesized nanoparticles (%)	Scavenging activity for Ascorbic acid (%)
50	39	80
100	48	82
150	65	87
200	72	90

advances for modified Ag-NPs as opposed to use of established methods that are closely combined and harmful to the environment. The present study reports the formation of biologically active Ag-NPs synthesized by a cost effective and natural synthetic method from *Coriander sativum* extract containing macromolecules like lignin, glycosides, aldehydes and phenolic compounds with effective antimicrobial, antioxidant and anticancer abilities. Synthesis of nanoparticles following irradiation by sunlight; a free bioenergy resource with acceptable reduction times had been documented in previous studies (Karimi Zarchi et al., 2011). The reduction of silver ions by the plant extracts is marked by the appearance of yellow–brown color solution reflecting the formation of Ag-NPs. SEM images showed that nanoparticles are spherically shaped and measure between 67 and 86 nm influenced by the parting of colloidal particles of distinctive sizes and shapes in relation to added substances including time of incubation and pH (Ibrahim et al., 2020). Also, zeta potential analysis validated the steadiness of silver nanoparticles. Electrical charges on the nanoparticles surface avoid agglomeration, thereby providing nanoparticles with stability. Zeta potential distributions of nano silver and average size of nano silver prepared with aqueous CS extract suggest that silver nanoparticles are highly stable. The EDX analysis verified the existence of silver nanoparticles and generally showed high signal energy peaks in the range of 2–4 keV for silver atoms.

Antimicrobial tests showed positive results for the obtained silver nanoparticles. Silver nanoparticles displayed antibacterial activity against multiple bacterial strains and demonstrated a strong zone of inhibition. The data obtained are in accordance with previous studies (Ashraf et al., 2019; Luna et al., 2016). The antibacterial activity of these AgNPs may be attributed to the generation of oxidative stress, disruption in replication of DNA, and or AgNPs can directly cause lysis of bacterial cell by damaging the cell membranes (Jones and Hoek, 2010). Earlier, different polyphenols and plant extracts was assessed for antimicrobial activity in pharmaceuticals and foods (Abdalla et al., 2020; Abdel-Shafi et al., 2019). Many phenol compounds present in coriander, rosemary, thyme, hops, sage, tea, cloves, and basil show antimicrobial activity against foodborne pathogens (Barbinta-Patrascu et al., 2013; da Silva et al., 2015) and further elucidation on the mechanism of action are needed. Yet, the antimicrobial activity could be attributed to the occurrence of multitude of phenolic compounds found in a single extract of plants. The antibacterial effects of silver nanoparticles also could be due to interaction of nanoparticles with putative peptides which are essential for cell viability and division.

The findings of the MTT assay on MCF-7 cells showed strong proportionality to the dose of silver nanoparticles capped with CS. The cytotoxicity increased as the concentration of synthesized nanoparticles increased. The observed *in-vitro* anticancer activity is indicative of Ag-NPs 'function as effective therapeutic agents in treating cancer. In addition, the synthesized Ag-NPs may be used as a catalyst for future applications such as bioindicators, sensing, nanomedicine growth and targeted drug delivery. The data obtained are in line with previous reports (Vivek et al., 2012; Patra et al., 2019). The cytotoxic property of the AgNPs could be primarily because of the uptake and penetration of these extremely nanosized AgNPs into the cell and intracellularly thereby damaging cell organelles and their function. Additionally, the electrostatic interaction between cells and AgNPs can also result in the destruction of the infected cells (Patra et al., 2019). The observed antioxidant ability of CS-AgNPs could be attributed to the capping of silver nanoparticles with phytochemicals flavonoids with several hydroxyl groups present in the CS extract. An imbalance between the pro-oxidants and antioxidant results in generation of oxidative stress in biological systems with decrease in antioxidant enzymes leading to damage of vital biomolecules and other cellular components. The exhibited scavenging effect of CS-AgNPs could be attributable to the combined effect of silver ions and above-mentioned phytochemicals via mechanism of hydrogen atom and single electron transfer reactions (Prior et al., 2005). Thus, it can be concluded that the green synthesized CS-AgNPs demonstrated enhanced antibacterial, anticancer, and antioxidant activity. Absence of usage of toxic chemical in green synthesis of these nanoparticles possibly extends its applications to biomedical, electrochemical and environmental fields.

5. Conclusion

Nanotechnology is the most important field for developing new medical applications. The present investigation is highly necessitated to throw more light upon the silver nanoparticles synthesized from medicinal plants to investigate the active principle action for biochemical and molecular studies. The green approach for Ag-NPs synthesis using biorenewable materials appears to be promising, as they need non-toxic chemicals to minimize silver salt. Significantly, biosynthesized Ag-NPs display a broad range of resistance to antimicrobials, and there anticancer activity represents promising antimicrobial agents with possible biomedical applications.

CRediT authorship contribution statement

Roua Alsubki: Methodology, Writing - review & editing. **Hajera Tabassum:** Data curation, Methodology, Writing - review & editing. **Manal Abudawood:** Data curation, Writing - review & editing. **Ali A. Rabaan:** Data curation. **Sabah Ansar:** Conceptualization, Writing - original draft. **Sarah F. Alsobaie:** Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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