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Antioxidant activity and cytotoxic evaluation of phytofabricated silver nanoparticles of Fig (*Ficus mollis* Vahl)

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Abstract

The present study aimed to evaluate Antioxidant and Cytotoxic activity of phytofabricated silver nanoparticles (*FmF*-AgNPs) derived from Figs of *Ficus mollis*. This green synthesized *FmF*-AgNPs were tested for antioxidant activity with DPPH assay and cytotoxicity activity against MCF-7 (Human breast adenocarcinoma cell lines) with MTT assay at various concentrations. The data obtained demonstrated that *FmF*-AgNPs posses both antioxidant activity and cytotoxicity activity which is dosage-dependent. In conclusion, results obtained revealed the potent therapeutic value of phytofabricated silver nanoparticles (*FmF*-AgNPs) can act as potent antioxidant and anticancer agent.

Keywords

Ficus mollis; *FmF*-AgNPs; MCF-7; DPPH Assay; MTT assay.

Introduction

Silver nanoparticles (AgNPs), due to their unique properties, promising features, and multifaceted medical applications, are being widely used in cancer therapy. AgNPs causes the apoptosis of cancer cells through caspase-dependent and mitochondrial-dependent pathways (1-3). Plant bioactive components used for the biosynthesis of green Silver Nanoparticles (AgNPs) can show the new ways for the progress of novel strategies for the treatment of various cancers due to their synergistic effect (4). These plant metabolites also stabilize the nanoparticles by capping them (5-7).

Phytofabricated silver nanoparticles were synthesized with an eco-friendly method, utilised to obtain silver nanoparticles mediated by plant parts as a precursor and natural ingredients. These plant mediated nanoparticles were synthesized by the bioreduction of metallic ions as its scalability is easy and can function as reducing, capping and stabilizing agents (4,6). The earlier reports from various studies also revealed that green synthesized silver nanoparticles which are having size < 50 nm can easily diffuse, deep infiltration of biomedical importance and improved accumulation can results in complete eradication of cancer cells or tumorogenic cells (8-15). It was reported that AgNps cause cytotoxic effect of depending on the concentration, dose, time and size. The Physico chemical interaction between the macromolecules of cancer cells and AgNps lead to cancer cell toxicity which result in generation of ROS activate Capase 3, induce DNA fragmentation and decrease the metabolism of cancerous cells leading to cell death (16,17). Plant derived nanomaterials and synthesis of silver nanoparticles has been widely demonstrated in the scenario of mining novel ma-

terial context for potential antioxidant and anticancer agents (18,19).

Ficus (Moraceae) species are tropical flagship plant species which are cultivated and considered as asthetic, used in religious rituals which has great medicinal properties, several therapeutic applications with no side effects and pertain plentiful ethnobotanical claims (20, 22). Fruits of *Ficus* spp. are termed as "Figs" which posses the varied and potent therapeutic compounds which confer antioxidant potency and other medicinal uses (23-27). Figs are present throughout all seasons are widely consumed by many organisms especially Birds, Bats, Reptiles. This interesting aspect aimed to focus on the figs for its biological therapeutic evaluation.

Our earlier study reported the phytosynthesis of AgNPs using *Ficus mollis* Vahl fruit extract, which acted as both a reducing and stabilizing agent for AgNPs and also Characterization of *FmF*- AgNPs was achieved using a range of spectroscopic methods (20-22). This phytosynthesized *FmF*-AgNps are spherical, face-centered, cubic, and crystalline and well-dispersed with average range size of 23 to 30 nm. SEM with EDAX studies revealed 23-38 nm (in 500 nm resolution) and TEM studies displayed 20 nm AgNPs without any agglomeration with spherical shape with size ranged from 14 to 18 nm (in 50 nm resolution). (20-22)

Owing to the diverse application of plant mediated synthesis of nanoparticles, this study was designed to assess the Antioxidant and cytotoxic activity of silver nanoparticles obtained through *Ficus mollis* fruit extract (*FmF*-AgNps*)*. Hitherto, no study was conducted on *Ficus mollis* fruit on this domain which we focused to investigate the therapeutic biological activity in this research.

Materials and Methods

In this present investigation, Aqueous extract and green synthesized silver nano particles (*FmF*-AgNps) using figs of *Ficus mollis* Vahl (Moraceae) were used as testing material for determination of Antioxidant potency and cytotoxicity activity. (20,21)

Determination of Antioxidant activity

DPPH Assay

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity is one of the commonly used standard rapid assay to assess the antioxidant potency of synthesized particles and compounds (28).

Briefly, the reaction mixture (3.0 mL) consisted of 1.0 mL DPPH in methanol and 1.0 mL of different concentrations of Aqueous extracts, *FmF*-AgNPs (25µg mL^{-1}, 50µg mL^{-1},75µg mL^{-1},100µg mL^{-1}) were vortexed. These samples was incubated for 30 minutes at room temperature and thereafter, the scavenging capabilities of the mixtures were measured as IC_{50} (Inhibitory concentration at 50% scavenging capability) and using UV-Spectrophotometer at 517 nm wavelength, the percentage of inhibition was evaluated as radical-scavenging capacity. Vitamin C was employed as standard control. The tests analysis was performed in triplicates, to determine the mean ± SD. Using the following formula, the antioxidant properties of DPPH free radical % is calculated for the samples.

$$\text{DPPH free radical Scavenging activity \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Determination of cytotoxicity

In the present ivestigation, anticancer activity of Aqueous extract and *FmF*-$AgNP_S$ was determined using calorimetric MTT assay (29) as followed with minor modification (15).

The MCF-7 cancer cells (Human breast adenocarcinoma cell lines) used in the assay were purchased from NCCS, Pune, India. The cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) high glucose media supplemented with 10% FBS (fetal bovine serum) along with the 1% antibiotic- antimycotic solution (Streptomycin) at a concentration of 5 X 10^3 cells/well in 96 -well plates and incubated in atmosphere of 5% CO_2, 18-20% O_2 at 37°C for 24hr in the CO_2 incubator to provide proper culture medium. After attaining 80-90% confluence, the cells were used for *in-vitro* experiments. After 24hr incubation, MCF-7 cancer cells were then treated with varying concentrations of test samples *FmF*-$AgNP_S$ (25 µg mL^{-1}, 50µg mL^{-1}, 75µg mL^{-1}, 100µg mL^{-1}, 150µg mL^{-1}). In further step, DMEM was removed and fresh MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; 5 mg/mL] (100µL) was loaded and the plates were kept in a incubator at 5% CO_2. Thereafter, finally MTT was added to the MCF-7 cells. 10µL of this solution was added into each well and incubated at 37°C for 4 hrs. Finally DMEM was replaced with 100 µL of DMSO (Dimethyl sulfoxide) solvent was added to each well and again placed into the incubator for 30 minutes. The conversion of MTT solution to formazen by surviving cells was measured as absorbance using microplate reader at a wavelength of 570 nm at 48 hr and IC_{50} concentrations were calculated. Doxorubicin is used as Positive control. The results were used to construct a graph of cell viability percentage against concentrations of fig aqueous extract, *FmF*-$AgNP_S$ and for standard. The percentage cell viability of testing sample was calculated by following formula :

$$\text{Percentage of cell viability} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100.$$

Results and discussion

Antioxidant activity

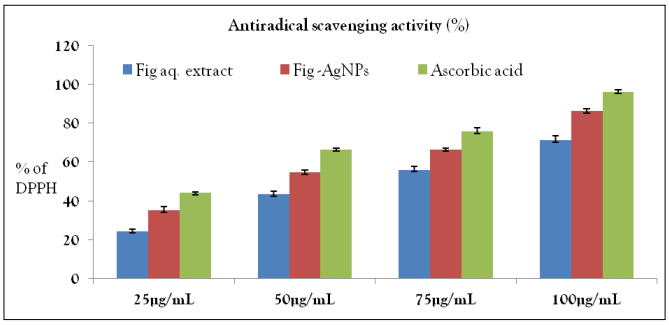
Antioxidant assay was performed evaluating the percentage scavenging by silver nano particles synthesized from *F.mollis* fig aqueous extract (*FmF*-AgNps) against DPPH radicals (20,21,22).

Results depicted an increase in the percentage and more active scavenging of radicals by Fig-AgNps (*FmF*-AgNps) compared to the aqueous *F.mollis* fig extract and

seems to be equal to the standard at higher concentrations. The radical scavenging potential seems to be increased while increase in the concentration showing dose-dependent character. This might be a possible prediction that chelation of the radicals on the large surface area of the nanoparticles. Table 1 and Graph 1 reports the scavenging percentage against the concentration. Fig-AgNPs percentage of scavenging capacity increased from (35.14±2.15% to 86.31±1.25 %) nevertheless the aqueous extract showed (24.39±1.12% to 71.13±2.40%) which is less capable when both are compared this could be due to the presence of Ag ion as opined by the other workers (28,30). The ability of aqueous extract of fruits of *Ficus mollis (*Aq. Figs) (IC_{50} = 64.59) was observed as lesser than *FmF*-AgNps but formed a good scavenging activity (IC_{50}=46.55). The presence of therapeutic phytoconstituents like Phenols, flavonoids, functional groups could be the reason for this property. IC_{50} (Inhibitory concentration at 50% scavenging capability) of the three attributes (Aq. Fig extract, *FmF*-AgNps and Standard –Ascorbic acid) are reported in Table.1.

Table : 1 : Antioxidant activity of Aqueous extract of Aq. Figs, *FmF* –AgNps & Standard.

Attribute	25µg mL^{-1}	50µg mL^{-1}	75µg mL^{-1}	100µg mL^{-1}	IC_{50} µg mL^{-1}
Aq. Fig	24.39±1.12	43.36±1.58	55.99±1.77	71.13±2.40	64.59
FmF -AgNps	35.14±2.15	54.55±1.31	66.45±0.65	86.31±1.25	46.55
Ascorbic acid	44.1±0.46	66.45±0.97	75.74±1.97	96.142±1.07	31.35



Graph :1 DPPH free radical scavenging activity of synthesized silver nanoparticles.(*FmF*-AgNps)

The order for the scavenging ability of the test samples : Standard (Ascorbic acid) (IC_{50}= 31.35; y = 0.7827x + 17.7; R^2 = 0.9976) > *FmF* -AgNps (IC_{50}=46.55; y = 0.7179x + 21.883; R^2 = 0.9857) > Aq. Figs (IC_{50}= 64.59; y = 0.6916x + 8.0333; R^2 = 0.9141).

Phytosynthesized nanoparticles using dried fruit extract of *Ficus carica* reported potent antioxidants and proved that these nanoparticles can act as anticancer agent (31). *In vitro* antioxidant potency was evaluated in Fig peels and pulps with a chemical and chemometric approach (26,27). IC_{50} value measure antioxidant activity, a smaller the IC_{50} value the higher higher the antioxidant activity of the sample (32,33). Based on the IC_{50} value obtained for the samples it was evident that *FmF*-AgNps posses strongest antioxidant capacity than aqueous extract (Fig- 1). This study reported that these phytofabricated particles (*FmF*-$AgNP_S$) are capable of scavenging DPPH radicals in a dose dependent pattern.

Cytotoxic evaluation

The biocompatibility of *FmF*-AgNPs with MCF-7 cancer cell lines was expressed as the percentage of relative viability of when compared to the untreated control cells (Table 2; Fig. 1). Results demonstrated that, *FmF*-AgNPs showed potent cytotoxic activity against the MCF-7, verifying the cell viability at different concentrations (25 µg mL^{-1}, 50µg mL^{-1}, 75µg mL^{-1}, 100µg mL^{-1}, 150µg mL^{-1}). All the inhibitions were experimented in triplicates and compared with the standard. The aqueous fig extract and *FmF*-$AgNP_S$ showed similar trend but comparatively *FmF*-$AgNP_S$ displayed greater inhibition of MCF-7 at all concentrations (Table 2).

The positive control exhibited the highest toxicity. In comparision, there was an inverse relation between percentage cell viability of MCF-7 cell lines and the concentrations of *FmF*-AgNPs. As it was clearly observed that the percentage of cell viability decreases when the dosage of *FmF*-AgNPs increased.

An increase in the concentration of *FmF*-AgNPs decreased the percentage cell viability and showed an increase in the cytotoxicity against MCF-7 cancer cell lines. Absorbance value measured in IC_{50} (IC_{50} is the inhibitory concentration of cytotoxic agent necessary to kill one-half of the cell population). The amount of absorbance correlates with viable cell number (32,33). In this assay, the absorbance was converted to a percentage of cell growth inhibition. *FmF*-AgNPs (IC_{50} =33.59 µg/mL) was more potent than aqueous extract (IC_{50} = 48.5 µg/mL). *FmF*-AgNPs showing highest anti-cancer potency due to its low IC_{50} value (33.59). The effective cytotoxic activity of *FmF*-AgNPs was observed at 100 µL/ml (17.93%). It is clearly evident that the MCF-7 cancer cells completely dead after 72h and no viable cells were observed, which proves the efficacy of biosynthesized *FmF*-AgNPs as an anticancer agent.

In this research, MTT assay was opted for the cytotoxic studies which is a colorimetric method widely used to test cell viability (32,33,34,35). Considering various protocols and several modifications accordingly we have cross checked with recent literature (1,35). MTT assay is based on mitochondrial uptake and succinate dehydrogenase reduction of soluble, yellow, MTT tetrazolium salt to an insoluble blue MTT formazan product which is precipitated in uninjured cells (36,37).

However, it was established that the interaction between AgNPs and cancerous cells by phytochemicals such as Phenols and flavonoids (38,39,40). In our studies, Phenols and primary amines reported in our FT-IR spectroscopy which predicted to be the reason for this property (22). Phenolics are shown to inhibit the initiation and progression of cancers by modulating genes regulating key

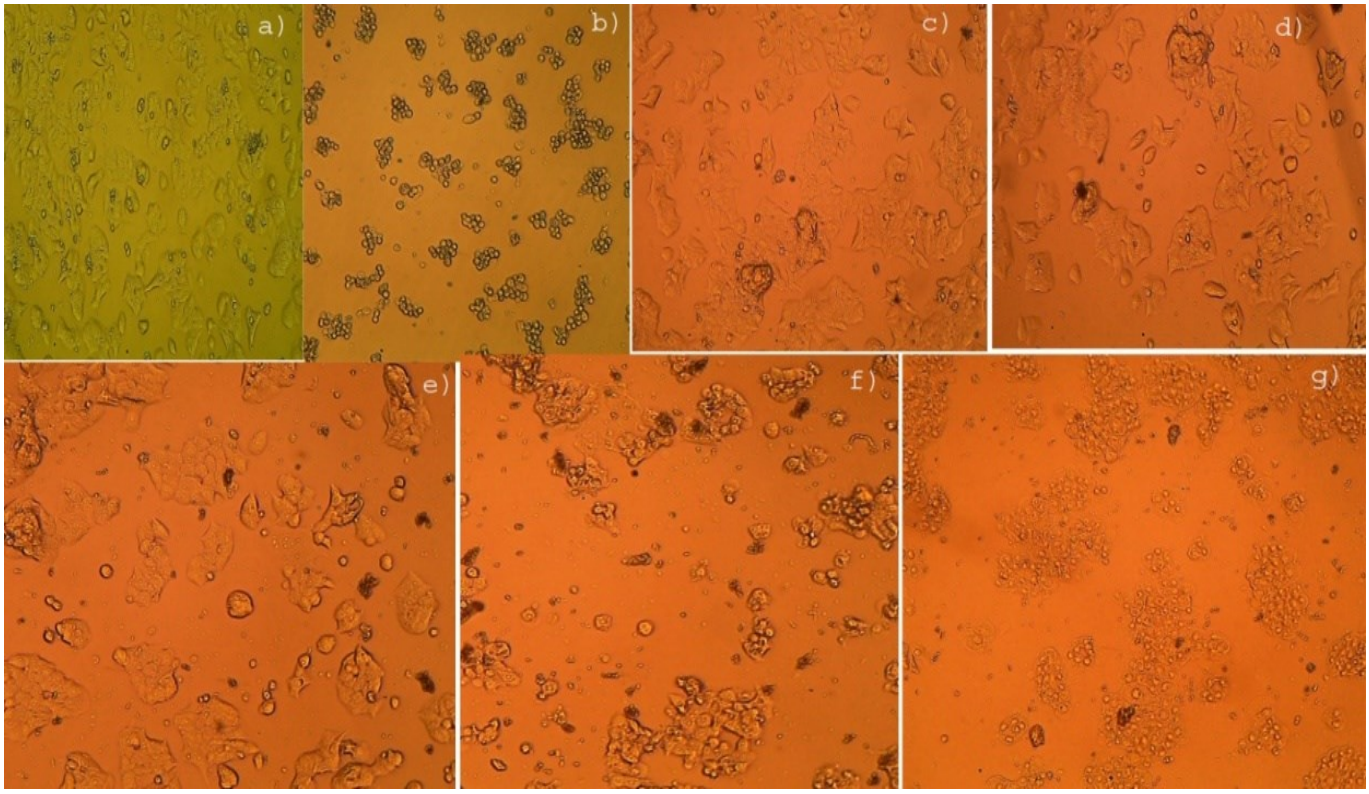


Figure 1. Representative images of cellular morphology showing Dose dependent Cytotoxic effect of *FmF*-AgNPs on MCF-7 cells and its Morphological changes observed under an inverted light microscope. **a**) Untreated **b**) Standard control (10µg/ml) **c**) 25 µL/mL **d**) 50 µL/mL **e**) 75 µL/mL **f**) 100 µL/mL **g**) 150 µL/mL

Table 2. *FmF*-AgNPs Treatment

Sample	25µg/mL	50µg/mL	75µg/mL	100µg/mL	150µg/mL	Std.control (10µg/mL)	IC_{50} µg/mL
Fig-AgNPs	93.79±0.54	80.91±0.48	61.93±1.46	36.15±0.30	13.93±1.24	42.71±1.35	33.59±1.22
Fig- Aq. Ex	97.05±0.22	86.64±0.56	76.44±0.62	50.65±0.84	27.03±0.66	45.26±0.68	48.5±0.52

processes such as angiogenesis and metastasis, oncogenic transformation of normal cells and proved to be a potent anticancer agent (41,42). It has been already proved that phenolic compounds constructed nanomedicine for innovative cancer treatment (43,44). Nanoformulation with the polyphenols may foster the technological process in health therapeutics (45). Finally, in this study it was expressed that *FmF*-AgNPs posses excellent cytotoxic efficacy against Human breast cancer (MCF-7) cells by using MTT assay.

Conclusion

In this research, it was demonstrated that the Nanoparticles synthesized from edible figs of *Ficus mollis* (*FmF*-AgNPs), posses potent antioxidant and anticancerous ability which could be recommended for further *in vitro* experiments in order to confirm the possibility of their application in the treatment of human melanomas.

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Authors contributions

KR carried out the research work and drafted the manuscript. NS designed the study and corrected the Manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: **:** Authors do not have any conflict of interests to declare.

Ethical issues: None

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