Bioengineered and biocompatible silver nanoparticles from *Thalictrum foliolosum* DC and their biomedical applications

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**Abstract**

Bioengineered nanoparticles display unique characteristics at the cellular, atomic, and molecular levels with their geometric shapes dictating suitable actions and use. In the present study, bioengineered and bio-compatible silver nanoparticles (AgNPs) were obtained from *Thalictrum foliolosum* leaf extract which served as a capping and reducing agent. The effects of different parameters, such as varying concentrations of silver nitrate (0.2, 0.5, 1.0, and 2.0 mM), leaf extract (24.5, 24, 23.5, and 23 mL), pH (2, 4, 6, 7, and 8), and temperature (20, 40, 60, and 80 °C) were examined on the synthesis of *T. foliolosum*-assisted silver nanoparticles (TF@AgNPs) and their characterization accomplished by UV–visible and Fourier transform infrared spectroscopy, X-ray diffraction, Transmission, and Scanning electron microscopy, and Zeta potential analyses. X-ray and microscopy results revealed that TF@AgNPs were spherical with ~18.27 ± 3.9 nm size. The biological studies indicate potential antifungal, antioxidant, and anticancer properties besides hydrogen peroxide sensing, for the ensued TF@AgNPs suggesting their numerous biomedical appliances. A clean, cost-effective, and safer method for the procurement of bioengineered TF@AgNPs that precludes the use of any hazardous elements with no adverse effects is some additional sustainable attributes.

**Graphical abstract**

**Keywords** Thalictrum foliolosum · Bioengineered · TF@AgNPs · Anticancer

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Introduction

Nanotechnology is a multidisciplinary and one of the most appealing research areas, as the use of nanoparticles (NPs) in various fields has increased extensively in recent years (Sharma et al. 2021). One-dimensional, small-sized (1–100 nm) NPs have a high surface-to-volume ratio and are highly reactive. The effect of this specific property of NPs, different from their bulk materials, has been observed in diverse disciplines, including chemical (Varma 2019), biomedical (Iravani and Varma 2020), agricultural (Nasrollahzadeh et al. 2020), and engineering (Lu et al. 2021) research. AgNPs have garnered consideration in the current decade because of their broad spectrum of applications in the environmental areas (Fiorati et al. 2020), sensing (Chandraker et al. 2019a), catalysis (Dong et al. 2015), optics (Rasmagin and Apresyan 2020), electronics (Fernandes et al. 2020), nanomedicine (Munenos et al. 2020), biomedical devices (Lyu et al. 2021), and antimicrobial (Algotiml et al. 2022) applications due to their unusual intrinsic physicochemical properties.

Among the two approaches, top-down, and bottom-up, the latter entails the assembly of single atoms or molecules to create NPs (Khandel et al. 2018) often via physical, chemical, and biological methods; biogenic approach, in contrast to physical and chemical methods, precludes the use and generation of hazardous materials (Zare et al. 2020). The ecofriendly greener synthesis of AgNPs has been accomplished using plant extracts, microorganisms, antioxidants, biosurfactants, and enzymes used as reducing and capping/stabilizing agents (Hebbalalu et al. 2013) which are economical and biocompatible processes (Moulton et al. 2010) and are ideal for large-scale processing.

Fungal diseases are severe health problems worldwide and effective therapies and management strategies must be increased (Scorzoni et al. 2017) via the production of low-cost and less toxic new antifungal drugs (Bocate et al. 2019). It has been reported that berberine present in the leaves of Thalictrum foliolosum has antioxidant and antifungal activity (Kumar et al. 2020). In this context, the synergistic effect of NPs and active ingredients would provide better results than drugs alone as affirmed by some previous findings (Mohsen et al. 2020).

Hydrogen peroxide (H₂O₂) has been a widely used green oxidant applied in various fields such as pharmacology, wood, food, textiles, electronics, wastewater treatment, cosmetics, tanning, and pulp industries (Chandraker et al. 2021a); global demand for the H₂O₂ market being 3,850 kilotons in 2015 is expected to reach ~6,000 kilotons by 2024 (Kim et al. 2018). However, the accumulation and even a minimal volume of H₂O₂ in the process can lead to numerous safety and environmental hazards due to its toxicity (Tagad et al. 2013). Therefore, accurate, stable, and expeditious methods for detecting H₂O₂ residues in commercially available goods are necessary among available means for sensitive detection of H₂O₂, namely spectrometric method, chemiluminescence, optical detection, electrochemical and batch-injection analysis (Hsu et al. 2015). There is potential scope to detect H₂O₂ using bioengineered AgNPs as has been suggested by the limited available studies (Rani et al. 2020).

The global burden of cancer is on the rise, and chemotherapy is one of the most widespread methods for the treatment of cancer. GLOBOCAN report from 2018, revealed 18.1 million cancer cases, with a death toll of 9.6 million (Bray et al. 2018). The advancement in nanomedicine provides a ray of hope and has made it an attractive option for cancer therapeutics because of its multifunctionality (Aghdabi-Maleki et al. 2020), as the nano-based systems are being studied for various applications, ranging from drug delivery to overcome drug resistance to imaging (Iravani and Varma 2021). The presence of cytotoxic alkaloids present in the leaves of T. foliolosum indicates that the AgNPs generated from them might be potent against cancer cells.

Thalictrum foliolosum DC. (Fig. 1) is a worldwide distributed, and perennial medicinal plant with ~200 species belonging to the family: Ranunculaceae with several diverse uses (Sun and Han 2019). Many plant extracts have recently been used to fabricate AgNPs (Salari et al. 2019; Khoda dadi et al. 2021). However, all plant-mediated AgNPs do not possess the same physical properties as well as the same chemical and biological activities; phytomolecules oriented on the surface of AgNPs determine their functionality. The varied properties of AgNPs are caused by phytochemical
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variations in different plants. The present study was therefore undertaken to bioengineer, optimize, and characterize silver nanoparticles (AgNPs) from T. foliolosum leaf extract (TFLE) and evaluate their antifungal, \( \text{H}_2\text{O}_2 \) sensing, antioxidant, and anticancer properties.

**Materials and methods**

**Collection of plant material**

Fresh leaves of *T. foliolosum* were obtained from Kapilhara site of Amarkantak, MP, India, in January 2019, duly authenticated by a taxonomist, and a herbarium specimen (Dob/10/TF/98-2019) was submitted to the Department of Botany, IGNTU, Amarkantak (India).

**Chemicals**

Himedia (Mumbai, India) provided silver nitrate (AgNO\(_3\)), \( \text{H}_2\text{O}_2 \), potato dextrose agar (PD-A), potato dextrose broth (PD-B), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS). All the chemicals used were of AR grade. All the equipment was cleaned with double distilled water (DDW) and placed in an oven (Scientech).

**Formulation of plant extract**

The deionized water was used to wash the leaves of *T. foliolosum*. About 20 g of the leaves were weighed and cut into identical pieces. Sliced leaves were placed in a 750 mL borosilicate flask with deionized water (200 mL), and boiled for 25 min at 65 °C. The filtrate (TFLE) was collected using Whatman No. 1 filter paper and kept at 4 °C.

**Phytochemical investigation**

The main phytoconstituents of TFLE were identified with the slight modification of the following earlier described protocol Chandraker et al. (2020), the main components being starch, lipid, protein, tannins, alkaloids, saponins, glucosides, mucilage, cellulose, and pectin.

**Optimization of *T. foliolosum*-assisted silver nanoparticles (TF@AgNPs)**

Different reaction parameters like TFLE and AgNO\(_3\) concentration, pH, and temperature were optimized to obtain the maximum and stable synthesis of TF@AgNPs. To optimize the temperature, 1 mL TFLE was blended with 24 mL AgNO\(_3\) (0.1 mM) and incubated at 20, 40, 60, and 80 °C for 80 min. The same experiment was then repeated at varying pHs (2, 4, 7, 6, and 8) using 0.1 N HCl/NaOH and incubated at 28 °C for 80 min to optimize the pH. One mM AgNO\(_3\) (0.5, 1.5, and 2.0 mL) solutions were mixed with varying amounts of TFLE (24.5, 24, 23.5, and 23 mL) and incubated at 28 °C for 80 min to optimize TFLE concentration. To optimize AgNO\(_3\) concentration, 1 mL of different AgNO\(_3\) concentrations (0.2, 0.5, 1.0, and 2.0 mM) were added to 24 mL of TFLE and incubated at 28 °C for 80 min. The sample was analyzed deploying UV–visible absorption spectroscopy to confirm the optimum formation of TF@AgNPs.

**Physicochemical characterization of TF@AgNPs**

UV–visible spectroscopy (Shimadzu UV-1800) was performed to confirm the phytosynthesis of TF@AgNPs. To affirm the crystallinity of TF@AgNPs, XRD analysis was carried out applying Bruker D8 X-ray Diffractometer at 30 kV and 20 mA current with Cu K (\( \lambda = 1.54 \text{Å} \)). FTIR (Bruker, Germany. Model: Vertex 70) spectral studies were done with Potassium bromide at 0.5 cm\(^{-1}\) resolution. To determine the size and shape of NPs, ultramicroscopic images were obtained using a transmission electron microscope (Technai G20 FEI) operating with 200 kV, and a current of 104 beams. A scanning electron microscope (EVO 18; Carl Zeiss, Germany) attached to energy dispersive X-ray (EDS/EDAX) was used to study the elemental configuration and morphology of TF@AgNPs. The average size of the particles, the size distribution, and zeta potential were calculated (Anton Paar, Litesizer 500).

**Applications of TF@AgNPs**

**Antifungal activity**

The aflatoxin-producing and post-harvest food spoiling fungi *Aspergillus flavus* (MTCC No. 277) was selected to evaluate the fungi toxic potential of TF@AgNPs. A. flavus was secured from the Microbial Type Culture Collection and Gene Bank (MTCC) of the Institute of Microbial Technology (IMTECH), Chandigarh, India. The anti-proliferative test for the mycelia inhibition of treated fungi was performed using semi-solid PDA media, combined with varying concentrations of TF@AgNPs (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL). Finally, a spore suspension of 1 × 10\(^6\) CFU mL\(^{-1}\) was inoculated in a 2 mm well at the center of each plate. Similar plates except TF@AgNPs were kept as negative controls, whereas TFLE and AgNO\(_3\) solutions were tested in different plates as positive controls. The test was performed in triplicate and all the Petri dishes were kept at 30 °C for six days in a BOD incubator. The radial growth of the fungal colony was measured and the photographs were captured thereafter. Percent fungal inhibition was ascertained following percent inhibition of radial growth (IRG) as follows:
where $R_1$ is radial growth in control, and $R_2$ stands for radial growth in various treatments. Data were analyzed with mean ± SE.

**H$_2$O$_2$ sensing activity**

To detect the H$_2$O$_2$ sensing capability of TF@AgNPs, the colloidal solution of TF@AgNPs (3 mL) was carefully mixed with 20 mM H$_2$O$_2$ (1 mL). Before adding H$_2$O$_2$, the initial absorption spectrum of TF@AgNPs solution was recorded at 450 nm. UV–Vis spectroscope (Shimadzu UV-1800) was used to record the deviation in the characteristic peak after a periodic spell of 3 min.

**Antioxidant activity**

The antioxidant activity of TF@AgNPs was assessed using the DPPH and ABTS free radical inhibition tests, as described by Chandraker et al. (2019b). The reduction in stable free radicals DPPH and ABTS by TF@AgNPs was determined following UV–visible spectroscopy, and the results were compared with reference antioxidant ascorbic acid. The percentage scavenging activity was calculated using the following formula:

$$\% \text{scavenging activity} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

(2)

**Anticancer activity**

Cancer cell lines A431 and B16F10 were cultured and stored at 38° C with 5% carbon dioxide in Dulbecco’s Modified Eagle’s (DME) medium containing 10% FBS and 1% penicillin–streptomycin–amphotericin B cocktail. The modified standard MTT assay of Singh et al. (2013) was used to determine the anticancer efficacy of TF@AgNPs against A431 (squamous cell carcinoma cell line) and B16F10 (melanoma cell line). Nanoparticles for treatment were dispersed in DMSO with the final concentration of DMSO being 0.1% in the medium. In a 96-well plate, both types of cells were seeded at a density of 5000 cells per well. After 24 h of seeding, cells were treated for 24 and 48 h with varying concentrations of TF@AgNPs (10, 25, 50, and 100 g/ml), after which the medium was gently switched with the MTT solution and incubated for four hours at 37 °C. To dissolve the ensued formazan crystals, 100 µL of DMSO was added, and the plates were further kept for 10 min at 37 °C. The absorbance was measured at 570 nm using a microplate reader.

The percentage cell viability was calculated with the following formula:

$$\% \text{cell viability} = \left( \frac{\text{Average absorbance of the treated sample}}{\text{Average absorbance of the control sample}} \right) \times 100$$

(3)

**Statistical analysis**

All of the experiments were repeated three times, and the results were calculated in mean ± SE using OriginPro 8.5 software. Data from the MTT assay were examined using the Student’s t-test, with $P < 0.05$ considered statistically significant.

**Results and discussion**

In this study, bioengineered TF@AgNPs were obtained from aqueous TFLE as reducing and capping agents, and their antifungal, H$_2$O$_2$, antioxidant, and anticancer properties were evaluated.

**Phytochemical analysis**

*Thalictrum foliolosum* is a well-known plant for its phytochemicals with an abundance of alkaloids (Bhakuni and Singh 1982). According to Ringmichon et al. (2013), the rhizomes of *T. foliolosum* contain starch, lipid, protein, tannins, alkaloids, saponins, glucosides, mucilage, cellulose, and pectin. In our study, most of these phytochemicals were confirmed in TFLE (Table 1) which can be coated on the surface of Ag+ and thus liable for the reduction in Ag+ to Ag0 and further preventing their agglomeration as corroborated by deployment of alkaloids (Almadiy and Nenaah 2018), tannins (Lakshmanan et al. 2018), and flavonoids (Sahu et al. 2016), among others. The Schematic representation for the reduction mechanism of TF@AgNPs is shown in Fig. 2.

**Optimization of TF@AgNPs synthesis**

The addition of TFLE to the aqueous solution of 2 mM silver nitrate resulted in a color change from colorless to a dark brown solution, as shown in the inset of Fig. 3. After 80 min at room temperature (28 °C) of reaction time, a signature surface plasmon resonance (SPR) band with λmax emerged at 450 nm. At the same time, no absorption spectra were observed in aqueous TFLE and AgNO$_3$ solution.
Optimization studies were performed to improve the yield of TF@AgNPs. Several experiments with different parameters, such as reaction times, the concentration of TFLE and silver nitrate, temperature, and optimal pH, were used to achieve a narrow size distribution of TF@AgNPs.

Phytosynthesis of TF@AgNPs outperforms the physical, chemical, and microbiological techniques by a wide margin. Green synthesis with aqueous plant extracts removes the need for costly machinery, chemicals, and microbial culture, uses less energy, and produces a better yield of NPs and environment-friendly by-products (Khandel et al. 2018). *T. foliolosum* is a perennial wild-growing herb of the Himalayan and Amarkantak regions, making it readily available and free of cultivation for deployment in the green synthesis of AgNPs.

| S. no. | Phytochemicals | Test | Result |
|-------|----------------|------|--------|
| 1.    | Alkaloids      | Mayer’s test, Wagner test, Dragendroff test | +ve, +ve |
| 2.    | Flavanoids     | Ferric chloride test and Lead acetate test | +ve |
| 3.    | Diterpenes     | Copper acetate test | −ve |
| 4.    | Tannins        | Ferric chloride test | +ve |
| 5.    | Glycosides     | Molish test | −ve |
| 6.    | Saponins       | Foam test | −ve |
| 7.    | Triterpenes    | Salkowski test, Liberman test | −ve |
| 8.    | Phlobatannins  | HCL test | +ve |
| 9.    | Steroids       | Salkowski test | −ve |
| 10.   | Cardiac glycoside | Keller kilanis test | −ve |

+ve, Present; and −ve, Absent

![Fig. 2](image-url) The schematic for the reduction in Ag⁺ to Ag⁰ and synthesis of TF@AgNPs
Effect of reaction time

Figure 3 shows UV–Vis spectra of bioengineered TF@AgNPs while their formation was monitored every 10 min. time intervals. Maximum reduction in and formation of AgNPs were observed and no impact on absorption spectra after 80 min. of reaction of AgNO₃ and TFLE. Even after one month, the TF@AgNPs showed an absorption peak of 450 nm thus affirming their stability.

Effect of temperature

To evaluate the influence of temperature on TF@AgNPs synthesis, TFLE (1 mL) was mixed with 2 mM AgNO₃ (9 mL) in four different vials and incubated at varying temperatures from 20 to 80 °C. After this optimization study, it became clear that raising the temperature of the reaction mixture increases the synthesis of bioengineered NPs; the maximum synthesis of AgNPs was observed at 80 °C Fig. 4a. There was a positive impact of temperature on the reaction mixture because high temperature increased the kinetic energy of molecules present in the solution and led to a faster synthesis rate (Saxena et al. 2016).

Effect of pH

During the synthesis of NPs, the reaction mixture’s pH plays a significant role; even the color of the reaction mixture, the intensity of the SPR peaks, shape, and size of the nanoparticles are pH-dependent. In our study, the acidic pH 2 and pH 4 show a low absorption peak at 450 nm compared to pH 6 and 8. The alkaline pH 8 was found optimal for the synthesis of NPs (Fig. 4b). According to Vanaja et al. (2013), the absorption increases with the rise in pH, also indicating that the alkaline medium gives better results than the acidic medium for NPs synthesis. A change in pH affects the ability of the biomolecules contained in the extract to neutralize and reduce Ag⁺ during NP production, which can sometimes impact the shape, size, and yield of NPs. Handayani et al. (2020) observed comparable results on an alkaline medium with Pometia pinnata (Matoa) leaf extract and supported the formation of stable NPs. Similarly, Qian et al. (2013) found that alkaline pH favored the synthesis of spherical and 10–15 nm AgNPs using an endophytic fungus (Epicoccum nigrum). According to Sintubin et al. (2009), higher pH causes more competition between protons and metal ions for patching bonds with negative charge areas; thus, the alkaline pH gave better results than the acidic pH for NPs synthesis.

Effect of TFLE concentration

Another vital factor for the greener synthesis of NPs is the concentration of leaf extract. The UV–Vis absorption spectra of TF@AgNPs were prepared using varying concentrations of TFLE (0.5, 1, 1.5, and 2 mL), where the AgNO₃ concentration was kept constant at 2 mM. Figure 4c depicts that in 0.5 TFLE concentrations, there is no SPR peak. After increasing the concentration of TFLE, SPR peaks were proportionally enhanced, the maximum peak intensity is observed at a TFLE dose of 2.0 mL. The synthesis of bioengineered NPs was enhanced by the increased concentration of biomolecules involved in metal reduction as has been reported earlier for the Pongamia pinnata leaf extract (Priya et al. 2016) and the Citrullus lanatus fruit rind extracts (Ndikau et al. 2017).

Effect of AgNO₃ concentration

To find the impact of the substrate on NPs synthesis, different concentrations of AgNO₃ solution (0.2, 0.5, 1, and 2 mM) were used to synthesize TF@AgNPs and their formation was monitored by UV–Vis spectral analysis thus evidencing the correlation between concentrations of AgNO₃ and synthesis of TF@AgNPs. In our study, a low absorption peak for TF@AgNPs was found at lower concentrations up to 1.0 mM, presumably due to the very less availability of Ag⁺ in the reacting solution. Figure 4d demonstrates that as the concentration of AgNO₃ was increased, the amount of TF@AgNPs synthesis increased as well. The UV–Vis spectrophotometer displayed significant absorption spectra at 450 nm with 2 mM AgNO₃, thus, thought to be the optimum concentration for the phytosynthesis of AgNPs. Using Vaccinium arctostaphyllos extract, Khodadadi et al. (2021) also observed the same pattern on raising the AgNO₃ from 0.5 to 10 mM. Sobczak-Kupiec et al. (2011) noticed an ideal nucleation rate of the silver particles and a progressive increase in absorption strength when increasing
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The AgNO<sub>3</sub> concentration from 100 to 2000 ppm. At high concentrations, however, the SPR peak broadens and moves to a higher wavelength, indicating the increased particle size and agglomeration.

**Characterization of TF@AgNPs**

**XRD analysis**

The X-ray diffraction was performed to confirm that the TF@AgNP formed were crystalline (Fig. 5). The TF@AgNP metal formed in our study show the form of fcc crystalline lattice as evidenced by the peaks at 2θ = 38.24, 44.33, 64.49, and 77.49 which were representing the (111), (200), (220), and (311) Bragg’s reflections of the face-centered cubic structure of silver. A comparison of the XRD data with the standard data confirmed the crystalline nature, and these planes correspond to the standard JCPDS file no. 04-0783 (Pattanayak et al. 2017).

**FTIR spectral analysis**

FTIR analysis was performed for the detection of functional groups which are present in the THLE and the surface of Ag<sup>0</sup>. The wavenumber and interpretation of probable functional groups of TF@AgNPs, and leaf extract of *T. foliolosum* are shown in Table 1 in SI.

**TEM, SEM, and EDX analysis**

TEM images of TF@AgNPs are shown in Fig. 6a and b, which reveals the formation of isotropic spherical AgNPs,
the average particle size being 18.27 ± 3.9 nm. This corresponds to the particle size spectrum calculated from the UV–Vis spectroscopy SPR band, and XRD.

SEM analysis revealed the shape and structure of TF@AgNPs, confirming the formation of homogeneous TF@AgNPs (Fig. 6c and d). The composition of the elements in TF@AgNPs was revealed using energy-dispersive X-rays (EDX). At 3 keV, the EDX displayed the major elemental peak, indicating metallic silver in Fig. 7a. After the quantitative estimation, elemental Ag was recorded with the highest weight percentage, i.e., 84.17%, whereas, O, Si, S, Cl, and Ca have 10.00, 0.63, 0.03, 4.74, and 0.43%, respectively (Fig. 7b).

Zeta particle size and potential

DLS measurements were carried out to actuate the size, distribution of particle, and zeta potential of TF@AgNPs in the aqueous phase. Zeta particle size was employed to determine the average size of TF@AgNPs, while zeta potential reveals the stability of bioengineered NPs in the aqueous medium. Figure 1a in the supplementary material shows that the corresponding average zeta particle size is 65.11 nm and polydispersity index (PDI) of 25.4. Because DLS measurements were based on the hydrodynamic radius of the particles in the aqueous medium, the particle size distribution was found...
higher than the average particle size of TEM. Figure 1b in the supplementary material shows that the corresponding zeta potential of TF@AgNPs is −21.0 mV, suggesting the stability of AgNPs. The capping of phytoconstituents, which are present in the TFLE, could explain the substantial negative potential value.

As per the findings, the size of AgNPs at optimum parameters was 18.27 ± 3.9 nm, with a range from 14.29 to 24.21 nm (Fig. 6a, b). The DLS evaluated size is substantially bigger than the TEM size, as expected. The variations in size may be attributable to the fact that TEM only quantifies a number-based size and distribution of physical dimensions and does not include any stabilizing agent, whereas DLS measures the hydrodynamic diameter, including the diameter of the material as well as any atoms or particles mounted on the surface or moving with the AgNPs in solution (Cumberland and Lead 2009). According to Huang et al. (2007) when compared to TEM, the above ions or other corresponding particles make the material appear larger to the instrument. As a result, the hydrodynamic diameter is always higher than the TEM-estimated values. Nonetheless, hydrodynamic diameter is important to understand to optimize the size of NPs and their efficiency in the biological and chemical systems (Blanco et al. 2015; Singhal et al. 2011).

**Applications of TF@AgNPs**

**Antifungal activity of TF@AgNPs**

*Aspergillus* sp. are ubiquitous fungi mainly found in stored grains. Certain *Aspergillus* species produce toxins that can harm human and animal health and lead to hepatic, nephrotoxicity, immunosuppression, and carcinogenicity (Bocate et al. 2019). Following an incubation period of 6 days, the inhibitory effect of different concentrations of TF@AgNPs against *Aspergillus flavus* is shown in Fig. 8. In our study, we observed that the mycelial growth of *A. flavus* is directly dependent on the concentration of TF@AgNPs. Inhibition of radial growth (IRG) was increased with the increasing concentration of TF@AgNPs (Fig. 8). Figure 9 depicts a bar graph showing IRG of TF@AgNPs against the *A. flavus*. At higher concentrations of 1 mg/mL, NPs showed the highest (84.48%) IRG and at 0.0625 mg/mL, IRG was 37.17. Besides, the IRG was found to be 21.59, 11.26, 42.71, 48.42, and 55.39% against TFLE, AgNO₃, and 0.125, 0.25, and 0.5 mg/mL concentrations of NPs, respectively.

Our outcomes are important because the second major cause of invasive aspergilloses after *Aspergillus fumigatus* is *A. flavus*, and superficial infection is the most common cause of it (Hedayati et al. 2007). In a similar investigation, Sowmiya et al. (2021) found only 46% inhibition of *A. flavus* at 1 mg/ml *Corallocarpus epigaeus* fabricated AgNPs. Jaffri and Ahmad (2018) confirmed the antifungal activity of *Prunus cerasifera* fruit extract mediated AgNPs against *A. flavus*, while Bocate et al. (2019) described the effect of fungi-mediated AgNPs in different *Aspergillus* species. It can be concluded that bioengineered TF@AgNPs exert substantial in vitro antifungal activity against *A. flavus* which could be attributed to sufficient particle size and the capping and reducing agent used in the synthesis process, which originates from TFLE.

**H₂O₂ sensing properties**

The precise detection and quantitative analysis of H₂O₂ is an essential requirement in the processes of food sterilization, the production of pharmaceuticals, and medical devices (Neal et al. 2017). Therefore, it is imperative to design a consistent and efficient technique for detecting H₂O₂. In our study, the bioengineered TF@AgNPs were assessed with potential H₂O₂ sensing capacity. Using the UV–visible spectrophotometer, the initial SPR spectrum of diluted
TF@AgNPs solution (3 mL) was recorded. In the TF@AgNPs solution, 1 mL of 20 mM H₂O₂ was applied, and relative spectra were observed at frequent intervals as shown in Fig. 10. After the reaction with H₂O₂, dark brown color of TF@AgNPs Fig. 10 (vial i) gradually disappeared as shown in (vial iii) and finally became colorless after 21 min shown in Fig. 10 (vial iii). There were no changes in the color of the control set without H₂O₂ and SPR absorbance remains the same. The H₂O₂ sensing properties efficiency of TF@AgNPs is further compared with previously published AgNPs Table 2 in the supplementary material.

Saratale et al. (2019) have suggested that the generation of free radicals due to the reaction of AgNPs with H₂O₂ is responsible for the degradation of AgNPs. The probable
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The catalytic reaction of sensing H$_2$O$_2$ depends on the redox reaction. An electron shifted from the surface of reduced site TFLE capped TF@AgNPs to H$_2$O$_2$ producing an OH$\cdot$ radical, and one more electron shifted from H$_2$O$_2$ to an oxidized surface of the TF@AgNPs to generate HO$_2$$\cdot$ radical. Finally, when the H$_2$O$\cdot$ radical reacts with OH$\cdot$ radical, H$_2$O and O$_2$ are generated (Aadil et al. 2016). The following redox reaction mechanism summarizes the probable H$_2$O$_2$ decomposition reaction process by TF@AgNPs.

$$
\begin{align*}
H_2O_2 + Ag^0 & \rightarrow Ag^+ + OH^+ + OH^- \\
OH^- + H_2O_2 & \rightarrow HO_2^- + H_2O \\
HO_2^- + Ag^+ & \rightarrow HO_2^+ + Ag^0 \\
HO_2^+ + OH^- & \rightarrow H_2O + O_2
\end{align*}
$$

**Antioxidant capability**

The results of the DPPH and ABTS radical scavenging experiments, both of which are relatively quick and sensitive, confirmed the antioxidant potential of TF@AgNPs. DPPH is a well-known stable free radical having maximum UV–Vis absorption within the range of 515–520 nm. This organic nitrogen radical has purplish-blue color and converts into a colorless or pale-yellow when it reacts with a substance with hydrogen/electron donor and its absorbance decreases (Chandraker et al. 2021b). The frequency of the transition of color depends on the concentration and nature of the sample. The antioxidant capability of TF@AgNPs was dose-dependent as shown in Fig. 11a and b. The DPPH radical inhibition of TF@AgNPs for the concentration range of 31.25 to 500 µg mL$^{-1}$ was found to be around 85.05 ± 0.04 to 92.74 ± 0.02% with 75.20 µg mL$^{-1}$ IC$_{50}$ Fig. 11a. For the standard compound ascorbic acid in the same concentration range free radical inhibition was observed from 75.64 ± 0.08 to 98.94 ± 0.03% with 41.5 µg mL$^{-1}$ IC$_{50}$. Salari et al. (2019), and Vorobyova et al. (2020) reported similar DPPH free radical scavenging activities from Prosopis farcta fruit extract and black currant pomace extract AgNPs. Similarly, at 31.25 to 500 mg mL$^{-1}$ concentrations of TF@AgNPs ABTS radical inhibition was found to be 15.67 ± 0.30 to 80.67 ± 0.05% with 188.62 µg mL$^{-1}$ IC$_{50}$. However, at the same concentration, ascorbic acid showed 38.39 ± 0.05 to 88.09 ± 0.1% inhibition with 173.12 µg mL$^{-1}$ IC$_{50}$. Figure 11b.

**Anticancer activity**

In vitro anticancer activity of TF@AgNPs against cancer cell lines, A431 and B16F10 were determined by MTT assay. It is a colorimetric assay that detects cell viability.
by reducing the yellow color dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) with the mitochondrial enzyme succinate dehydrogenase to generate formazan crystals. The cell viability of both A431 and B16F10 cells was shown to be reduced after 24 and 48 h of treatment, and the viability of cancer cells was reduced in a concentration-dependent manner. TF@AgNPs at the concentration of 100 µg/ml caused ~20% and ~50% decrease in the survival of A431 and B16F10 cells, respectively, compared to control (Fig. 12). The decrease in cell viability was profound in the case of B16F10 cells where a significant decrease \( (P < 0.05) \) in the cell viability was observed. After 24 h of treatment, TF@AgNPs were shown to be more effective against B16F10 cell lines with an IC\(_{50}\) of 98.89 µg/ml compared to A431 cell lines with an IC\(_{50}\) of 231.37 µg/ml. AgNPs are known to produce reactive oxygen species (ROS), which can contribute to oxidative damage, and there are reports about induction of apoptosis through caspase 9/3 dependent pathway by AgNPs through ROS generation under in vitro conditions (Ahamed et al. 2010).

The cytotoxic effect of AgNPs could also be due to the inorganic NPs being taken up by the cell, triggering the signaling cascades, and their enhanced interaction with DNA and proteins (Franskevych et al. 2016). As per Kovács et al. (2022), AgNPs enter the malignant cell via an endocytotic process, triggering the production of intracellular ROS, which not only stimulates MAP kinase, disrupting the cell cycle but also targets genomic DNA for intrinsic damage. The activation of the tumor suppressor P53, which leads to apoptosis, occurs as a result of DNA damage. AgNPs increase oxidative stress, causing the antioxidant glutathione to be further oxidized to glutathione disulfide. Consequently, the ROS damages in cells are unavoidable. Additionally, AgNPs have been demonstrated to inhibit a damage-repair enzyme DNA-dependent protein kinase (Lim et al. 2017). Figure 13 summarizes the probable mechanism for the uptake of AgNPs and anticancer activity. However, for the detailed mechanisms, further studies are needed.

Additionally, alkaloids have also been isolated from T. foliolosum having cytotoxic activity against malignant cell

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**Fig. 11** Free radical scavenging activity of TF@AgNPs against DPPH (a), ABTS (b)

**Fig. 12** Percent viability of A431 and B16F10 cell lines after incubation (24 and 48 h) with varying concentrations of TF@AgNPs
lines such as A549, BGC823, Hep-G2, HL60, MCF7, and SW480 (Sun and Han 2019). Thus the secondary metabolites might also be contributing towards the enhanced cytotoxicity of TF@AgNPs. Furthermore, AgNPs can be used to deliver various cytotoxic medications or to improve the anti-cancer efficacy of attached chemotherapeutics. Hypotheses support the clinical trials of AgNPs integrated with nanoemulsions, cyclodextrins, hydrogel beads, and liposomes resulting in greater compatibility, reduced toxicity, and targeted delivery. (Ivanova et al. 2018). Biodiagnostics and targeted gene therapy may benefit from the coupling of AgNPs with bioactive compounds and oligonucleotides.

Conclusions

Bioengineered AgNPs were developed by deploying pharmacologically active Thalictrum foliolosum leaf extract via a cost-effective and eco-friendly strategy. TF@AgNP displayed the characteristic SPR peak of Ag$^0$ at 450 nm, and the maximum and stable synthesis were optimized at 2:8 ratio of leaf extract and AgNO$_3$ (2 mM) solution, pH 8, and 80 min. of incubation at 80 °C. XRD and microscopic studies revealed the face-centered cubic lattice and spherical shape of TF@AgNP with an average size of 18.27 nm. TF@AgNPs at 1 mg/mL showed 84.48% mycelial growth inhibition of aflatoxin-producing and food spoiling mold Aspergillus flavus. TF@AgNPs displayed DPPH and ABTS free radical inhibition like that of ascorbic acid with respective IC$_{50}$ values of 75.2 and 168.6 µg/mL. Explored antifungal and antioxidant activities of TF@AgNPs recommend their appropriate uses in food industries for active packaging to maintain improved food storage and enhanced shelf-life. TF@AgNPs were found to show potential cytotoxicity against mouse murine melanoma (B16F10) and human epidermoid carcinoma (A431) cell lines with 98.89 and 231.37 µg/mL IC$_{50}$ values, respectively. The findings revealed here lay the groundwork for future applications of these NPs in cancer treatment. The capability of TF@AgNPs to detect hydrogen peroxide within 21 min. renders them, suitable agents, for nano-remediation of pollutants and ROS. To our knowledge, this is the first investigation on the biological properties of Thalictrum foliolosum mediated AgNPs, which bodes well for their prospective application in biomedical sciences. The diverse and relevant outcomes of the synthesized TF@AgNPs and their generation via a green synthesis method hold promise in future biological studies with the appropriate change in the size and form of the metallic NPs.

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