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Research Article

Ascorbic Acid and Polyphenols Mediated Green Synthesis of Silver Nanoparticles from *Tagetes erecta* L. Aqueous Leaf Extract and Studied Their Antioxidant Properties

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Silver nanoparticle synthesis of the leaf extract *Tagetes erecta L.* enriched with ascorbic acid and polyphenols has been investigated. The color of the golden yellow extract has changed to pinkish-brown due to the reduction of Ag^+ to the colloidal solution of AgNPs and a sharp absorption peak at 420 nm under the UV-Vis spectrophotometer. In addition, the Fourier Transfer Infrared Spectroscopy (FTIR) estimation was completed in order to recognize and identify the biomolecules present in the extract acting as a reducing and capping agent for the AgNPs. The X-ray diffraction (XRD) peaks at (111), (201), (220), and (311) confirm the presence of monoclinic crystals in the solution. The morphology and size of the particles were provided by transmission electron microscopy (TEM) images of AgNPs. At a scale of 100 nm, synthesized AgNPs were predominantly spherical with a size range of 7-35 nm. In comparison to 7.39 mg/100 g in AgNPs, aqueous leaf extract was 55.14 mg/100 g higher in ascorbic acid. The phenolic and flavonoid content of extract was 52.54 ± 2.15 mg (GAE/100 g) and 15.43 ± 0.34 mg (QE/mL), and the colloidal AgNP solution was 21.45 ± 1.15 mg (GAE/100 g) and 8.05 ± 2.42 mg (QE/mL), respectively. Phenolic and flavonoid contents play a major role as a reducing agent and reduce the precursor $AgNO_3$ into AgNPs. The DPPH scavenging assay also assessed the antioxidant properties of extract and its derived AgNPs. As compared antioxidant value to aqueous leaf extract (mg/mL), higher percentage inhibition (PI) was found in AgNPs and free-radical scavenging activity of extract and AgNPs were directly linked to their concentrations. Results of this research have discovered a higher potential for free-radical scavenging AgNPs and will help to develop new and more potent antioxidants for the treatment of different diseases caused by oxidative stress; the higher antioxidant properties bearing AgNPs might be used.

1. Introduction

Today, a lot of novel protocols have been developed for silver nanoparticle (AgNP) synthesis with different shapes and sizes that has been attractive to nanotechnologist as well as biologist [1, 2]. With five hundred tons of silver nanoparticles produced per year, silver is one of the most commercialized nanomaterials [3] and is expected to increase in the next few years. Numerous methods of silver nanoparticle synthesis are available in the scientific community physical as well

as chemical in which the sol-gel process, chemical vapor deposition, chemical reduction, reverse micelle, microwave, hydrothermal method, laser-mediated synthesis, electroirradiation, microwave irradiation, ultraviolet (UV) irradiation, and photochemical reduction and biological methods from plants, microorganism, and yeast are common [4–8]. However, the synthesis of nanoparticles from materials (biological) such as plant leaves, stems, roots, and flowers is preferred due to their eco-friendly nature, cost-effectiveness, and less involvement of toxic chemicals for scientific community [9–11]. Therefore, the higher reduction rate and better stability of silver ions were observed in the extract of plant leaves as compared to microorganisms [11, 12]. Literatures available on different platforms regarding silver nanoparticle synthesis from biological material include plant leaf extracts from *Azadirachta indica* [13], *Cassia tora* [14], *Dracocephalum moldavica* [15], *Elephantopus scaber* [16], and *Ziziphora tenuior* [17]. The various parts of the plant extract [18, 19], black tea leaf [20–22], seeds like Mucuna pruriens [23], citrus fruits (Citrus limon, Citrus reticulata, and Citrus sinensis) aqueous extract of mushroom-mediated green synthesis [24, 25], and also flowers like Nyctanthes arbortristis [26] play a role as reductase and reduction of silver ions into silver nanoparticles [27]. Further, the application of silver nanoparticles is not limited to one area only [28]. For example, silver nanoparticles can act as antibacterial agents against pathogen bacteria such as *S. aureus*, *L. monocytogenes*, *E. coli*, *S. enterica*, and *P. fluorescens* [9, 11]. Moreover, silver nanoparticles can be seen to play an important role in the remediation of multiple pollutants [8, 29]. Despite the recent advances in the field of nanobiotechnology, we have not been able to establish exactly how different bioactive compounds in plants contribute in nanoparticle synthesis and why different plants exhibit different levels of nanoparticle synthesis even after applying the same protocol for the synthesis. One hypothesis for this is that each species has different levels of bioactive compounds and as some of these contribute greatly in nanoparticle synthesis, it is important to figure out their exact role in nanoparticle synthesis so that a standardized protocol for a large scale production could be developed which is a currently major drawback associated with biosynthesized nanoparticles that the amount of synthesized nanoparticles varies every time.

Hence, the aim of the present study was to investigate a rapid, simple, and eco-friendly approach for controlled-sized silver nanoparticle synthesis using the extract of the leaf parts having a good amount of polyphenol and ascorbic acid of *Tergate*. Further, the role of phytochemical involved during the synthesis of silver nanoparticles was identified. In addition, this morphological characteristic of silver nanoparticles was examined through techniques like UV-Vis spectra, TEM, FTIR, and XRD. Further, the antioxidant activity of silver nanoparticles was examined for their potential application in the healthcare sector.

2. Materials and Methods

2.1. Materials and Reagents. The healthy leaves of *Tagetes erecta* were collected from the botanical garden of this institute. Analytical grade reagents used in the study including 2,6-dichlorophenolindophenol (DCPIP or DPIP) or blue dye, α,α-diphenyl-β-picrylhydrazyl (DPPH), standard L-ascorbic acid, 20% glacial acetic acid, Folin-Ciocalteu reagent, sodium bicarbonate, methanol, and silver nitrate were purchased from HiMedia Laboratories and Sigma-Aldrich.

2.2. Preparation of the Plant Extract. 10 g leaves of plant were taken and thoroughly washed in distilled water and then crushed using mortar pestle. After crushing, the leaves mixed into 100 mL of deionized water followed by boiling for around 10 min at 60°C. The aqueous leaf extract was filtered through a Whatman filter paper (No.1), centrifuged for up to 10 min at 5000 rpm. The supernatant was stored at 4°C for further applications.

2.3. Preparation of the Dye and Standard Ascorbic Acid for Determination of Vitamin C. The dye solution was prepared by dissolving 100 mg of 2,6-dichlorophenolindophenol (blue dye) in 100 mL of distilled water. The mixture was diluted into 4 folds, filtrates, and was stored at 4°C for further use. 100 mg standard L-ascorbic acid dissolved in 50 mL of 20% glacial acetic acid and diluted to 100 mL with distilled water.

2.4. Determination of Vitamin C by the Titration Method. 10 mL of the L-ascorbic acid solution was titrated with the blue dye solution. Each drop of the blue dye in contact with the L-ascorbic acid solution turns pink in color. The endpoint was reached when the pink color lasts for 15 seconds [30, 31]. Similarly, 10 mL sample of *Tagetes erecta* prepared was in turn titrated and the titre values were recorded.

2.5. Determination of Phenolic Content. The amount of phenolic content in the extracts of *Tagetes erecta* was evaluated by Folin-Ciocalteu (F-C) assay as previously described by Velioglu et al. with some modification [32]. 200 μL aqueous leaf extract sample was mixed with 1.5 mL of 10 times diluted F-C reagent. 1.5 mL of 6% (w/v) sodium bicarbonate was added after 5 min in the solution for neutralized purposes. The solution was kept 90 min in dark and further takes an absorbance at 760 nm. Phenolic contents were calculated by using the Gallic acid equivalent standard curve. Results were expressed as mg/mL of Gallic acid equivalent (GAE).

2.6. Determination of Flavonoid Content. Flavonoid content in the leaf extract of *Tagetes erecta* was determined through the colorimetric method as described by Woisky and Salatino with some modification [33]. 0.5 and 1 μL/mL aqueous leaf extract was mixed with 1.5 mL of ethanol with 0.1 mL (10% aluminum chloride) and 2.8 mL of dH_20. The mixed reaction solution was shaken at RT for 20 min, and then, absorption was monitored at 415 nm. Quercetin equivalent was used as a standard. The amount of total flavonoid was expressed as quercetin equivalent (QE).

2.7. Green Synthesis of Silver Nanoparticles. Silver nanoparticle synthesis from leaf extract of *Tagetes erecta* was determined by the biological method as describe by Tyagi et al. with some modification [34]. 1 mM liquid silver nitrate ($AgNO_3$) was ready in 100 mL deionized water. Aqueous leaf

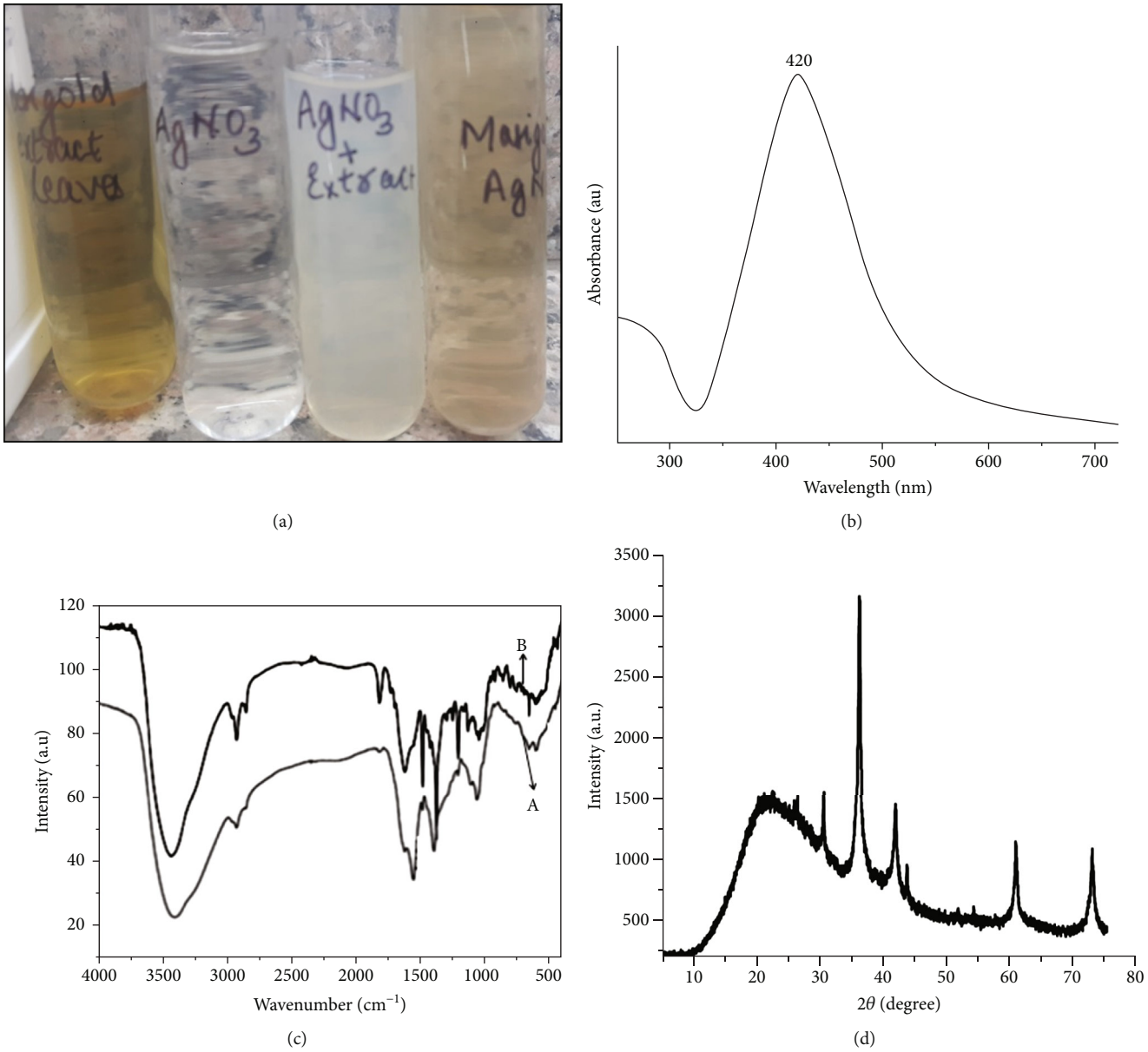

Figure 1: Visual color changing process from extracts to AgNP formation of *Tagetes erecta L.* (a), UV-Vis spectra analysis of AgNPs (b), FTIR spectra comparative analysis of *Tagetes erecta L.* and AgNPs (c), and X-ray diffraction pattern of the AgNPs (d).

extract (90 mL) of *Tagetes erecta* was added drop by drop in a flask bearing with 10 mL of liquid solution silver nitrate (1 mM), and pH was adjusted to near 7.2. The solution containing a flask was kept under direct sunlight for up to 30 min and wait for reduction from Ag^+ ion to colloidal Ag^0. The formations of AgNPs were confirmed by color changing from golden yellow to pinkish-brown. Primary confirmed synthesized AgNPs were purified through centrifugation process at 10,000 rpm for 25 min and again redispersed into deionized water for eliminating the water-soluble residues.

2.8. Determination of Antioxidant Properties. Antioxidant properties were determined by using α,α-diphenyl-β-picrylhydrazyl (DPPH) as a free-radical scavenging assay described by Chang et al., after some modification [35]. Dissolved 1.9 mg DPPH in 100 mL methanol and shake for 10 min or up to DPPH dissolved in solvent. DPPH (2.97 mL) was added to the different concentrations of samples (200, 400, and 600 μg/mL) prepared in methanol. The reaction solution was kept at RT for 20 min in darkness. The absorbance of the reaction sample was monitored under UV-Visible at 517 nm. Here, DPPH was used as the control sample. The PI value of scavenging was determined as follows: $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/(\text{Absorbance}_{\text{control}})] \times 100$. Mean value (M) and standard error (SE) were calculated by performing the experiments three times.

2.9. Characterization of Silver Nanoparticles. Characterizations of AgNPs were done with the help of different nanotechnology techniques. Primary confirmation of AgNPs was

Table 1: Peak position and assignment of main IR absorption bands of aqueous leaf extract and silver nanoparticles.

S. No.	Sample	Peak position wavenumber (cm^{-1})	Peak assignment
1	Aqueous leaf extract	3415	O-H stretching (alcohol & phenol)
		2922	C-H stretching (alkanes)
		1829, 1399, &1060	C-O stretching and C=O stretching (alcohol, ethers, carboxylic acids, and esters all have carbonyl functional groups)
		1643-1564	N-H stretching (amide linkages of proteins)
		1490	C=C stretching (aromatic compounds)
		670	O-H bend
		609	C-H bending
2	Silver nanoparticles (AgNPs)	3388	O-H stretching (alcohol & phenol)
		2933	C-H stretching (hydrocarbons such as alkanes & aldehydes)
		1829, 1399, & 1060	C-O stretching and C=O stretching of carbonyl bands shifted (clearly indicating the coordination of carboxylic acid with AgNPs)

based on color changes in the colloidal solution. Furthermore, confirmations of the synthesis of AgNPs, the absorption plasmon peak of the colloidal solution, were recorded under a UV-Vis spectrophotometer by scanning the spectra. The involvement of the functional group in Ag^+ reduction and applying formation of the phytofabrication aqueous leaf extract-mediated AgNPs were studied using FTIR. The size and shape of nanoparticles were analyzed under TEM. The XRD of AgNPs analyzed and determined the particle size by using the Debye-Schrrer equation.

3. Results and Discussion

3.1. Characterization of Silver Nanoparticles. Leaf extract of *Tagetes erecta* visual color golden yellow changes into pinkish-brown, this may be done to the reduction of Ag^+ from $AgNO_3$ into the Ag^0 in the colloidal solution of AgNPs, and a sharp absorption peak was obtained at 420 nm under a UV-Vis spectrophotometer. These color changes and sharp peaks confirm the biosynthesis of colloidal AgNP formation in the solution (Figures 1(a) and 1(b)). In some previous reports, a plasmon peak ranging 400-500 nm in AgNP formation [36] and some reports evident the absorption peak at 350-450 nm for the silver nanoparticles [27]. All in all, some factors such as size and shape of nanoparticles present in the reaction solution may be affecting the SPR absorbance.

The FTIR spectra of aqueous leaf extract and AgNPs synthesized from *Tagetes erecta* were compared. Aqueous leaf extract (A) and AgNPs (B) have FTIR peaks at 3415, 2922, 1829, 1643, 1564, 1490, 1399, 1060, 670, and 609 cm^{-1}, respectively. The O-H stretching vibration of alcohol and phenol compounds is assigned to the band at 3415 cm^{-1}, while the C-H stretching mode in alkanes is assigned to the band at 2922 cm^{-1}. The C-O stretching and C=O stretching modes of the carbonyl functional groups in alcohol, ethers, carboxylic acids, and esters are responsible for the FTIR bands observed at 1399, 1060, and 1829 cm^{-1}. N-H bending vibration of amides was attributed to the bands at 1643 and 1564 cm^{-1}. The C=C stretching mode in aromatic compounds is assigned to the bands at 1490 cm^{-1}. O-H bend and C-H bending are responsible for the peaks at 670 and 609 cm^{-1}, respectively (Table 1). The C-H stretching (alkanes) at 2922 cm^{-1} and the carbonyl bands at 1829 cm^{-1} were shifted to 2917 cm^{-1} and 1819 cm^{-1}, respectively, during the formation of AgNPs. The reduction of Ag^+ from $AgNO_3$ into Ag^0 thought biosynthesis process with the help of reeducates and capping material present within the leaf extract with the functional groups of the regions 2922 cm^{-1} (Figure 1(c)). FTIR often used to recognize and identify the biomolecules act as a reducing and capping agent for AgNPs. Both reduction and capping events contribute significantly in the process of AgNPs, and during this process, the various phytoconstituents such as alkaloids, amino acids, flavonoids, steroids, glycosides, tannins, and phenolic contents are involved for the synthesis and stabilization of AgNPs by forming different linkages with silver ions. The plant leaf extracts having a higher quantity of vitamin C and polyphenols contributed a significant role for the stable biosynthesis of nanoparticles. Changes within the intensity of FTIR peaks and small shifts were observed in the spectra of the extract and therefore the nanoparticles. This could ensure to the coordination of phytochemicals with the metal surface [37]. Necessary linkages like C=O, –C–OC–, N–H, –C=C–, –C = C–H, and C–H are formed during the process of nanoparticle synthesis [38]. Similar linkages were also observed in this investigation.

Synthesized AgNPs were studied through XRD for more analysis of its crystalline nature. Results of XRD represent the crystalline planes showing peaks and characteristics of nanoparticles (Figure 1(d)). The four main diffraction peaks for dried AgNPs were observed at 2 theta = 38.4, 44.5, 64.4, and 77.8, respectively; these peaks were corresponding to (111), (201), (220), and (311) planes of the face-centred cubic crystal structure of silver. Obtained results of XRD are in agreement with the previously published research confirming the cubic structure of silver [39, 40]. Similar XRD were recorded in Erigeron bonariensis [41] and Alternaria solani [42].

TEM pictures give more insight into the morphology, size, form, and distribution profile of the AgNPs. TEM

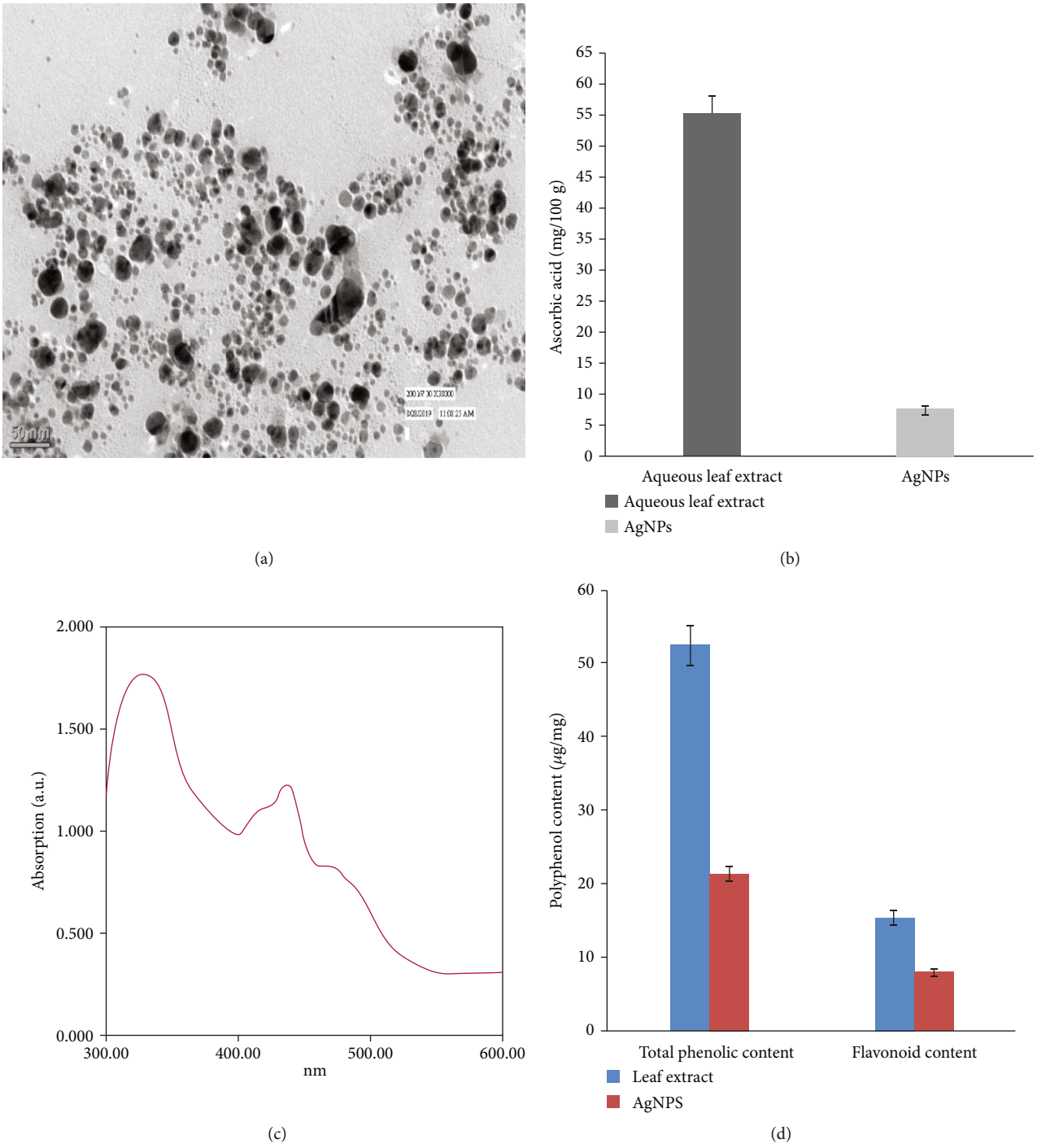

Figure 2: TEM results of AgNPs showing different sizes and shapes: (a) a comparative analysis of ascorbic acid (mg/100 g) between aqueous leaf extract of *Tagetes erecta L.* and AgNPs; (b) the absorption peak of aqueous leaf extract of *Tagetes erecta L.*; (c) the total phenolic and flavonoid content of aqueous leaf extract of *Tagetes erecta L.* and AgNP colloidal suspension.

pictures of AgNPs show the various shapes such as circular, rounded, triangle, and spherical, but mainly spherical in shapes with smooth surface nanoparticles were predominant. Synthesized AgNPs were predominantly spherical shape with the size range of 7-35 nm (average size of 22 nm) at 50 nm scale (Figure 2(a)). Biosynthesis of smooth and spherical nanoparticles has been reported in *Sida cordifolia* [37], *Aesculus hippocastanum* [43], *Rheum emodi* [44], and honey-mediated green synthesis of silver nanoparticles [45]. TEM pictures additionally indicated the formation of aggregates of AgNPs. In these aggregates, the particles were not directly connected to every alternative. This implies that the nanoparticles were stable by the aqueous leaf extract of *Tagetes erecta* that acted as a capping agent.

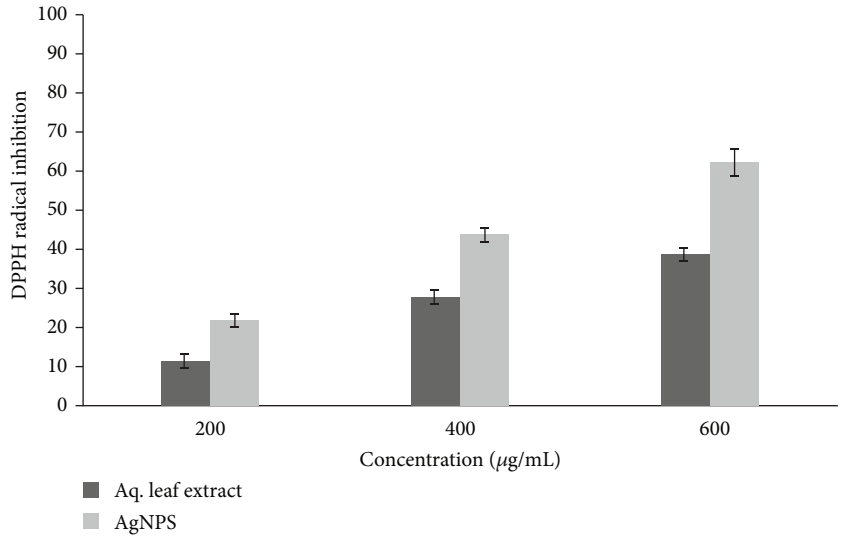

Figure 3: Antioxidant activity (% inhibition) of aqueous leaf extract of *Tagetes erecta L* and its derived AgNPs assessed by DPPH scavenging assay.

3.2. Estimation of Ascorbic Acid. Ascorbic acid was estimated in both *Tagetes erecta* aqueous leaf extract and its derived silver nanoparticles.

3.2.1. Ascorbic Acid in Extract. 6.5 mL of the 2,6-dichlorophenolindophenol (blue dye solution) was required to titrate 10 mL of the ascorbic acid (L-ascorbic acid) solution which contained 1 mg of ascorbic acid per mL.

That is, 6.5 mL blue dye solution is required to titrate, 10 mg ascorbic acid.

Therefore, 1 mL = (10/6.5) = 1.54 mg.

For *Tagetes erecta* aqueous leaf extract, the average mL of the blue dye used was 3.58 mL.

That is, 3.58 mL = 1.54 × 3.58 = 5.514 mg.

Therefore, 100 mL of the *Tagetes erecta* aqueous leaf extract contains 55.14 mg/100 g ascorbic acid.

3.2.2. Ascorbic Acid in Colloidal Solution of AgNPs. 10 mL colloidal solution of AgNPs was titred, 0.48 mL blue dye solution, and contains 0.739 mg ascorbic acid.

That is, 0.48 mL = 1.54 × 0.48 = 0.739 mg.

Therefore, 100 mL of AgNPs synthesized from *Tagetes erecta* contained 7.39 mg/100 g of ascorbic acid.

All in all, it is concluded that the higher amount of ascorbic acid content was 55.14 mg/100 g in *Tagetes erecta* leaf extract as compared to 7.39 mg/100 g in AgNPs (Figure 2(b)). The similar results were obtained in earlier reports in *Hibiscus rosa-sinensis* and *Citrus sinensis* [46], Chenopodium and Marigold [30], onion extract [47], and leaf extract of *Psidium guajava* [48].

3.3. Estimation of Total Phenolic and Flavonoid Content in Extract and AgNPs. The absorbance peak of *Tagetes erecta* aqueous leaf extract was observed at 370 nm, which is typical showing the presence of flavonoid (Figure 2(c)). The total phenolic and flavonoid content of *Tagetes erecta* aqueous leaf extract 52.54 ± 2.15 mg (GAE/100 g) and 15.43 ± 0.34 mg (QE/mL) and the colloidal solution of AgNPs 21.45 ± 1.15 mg (GAE/100 g) and 8.05 ± 2.42 mg (QE/mL) were estimated, respectively (Figure 2(d)). The obtained results conclude that the higher amount of total phenolic and flavonoid content was in the extract as compared to AgNPs. Total phenolic and flavonoid content aqueous leaf extract of *Tagetes erecta* may be played a role as a reducing agent and reduce the precursor $AgNO_3$ solution to colloidal AgNP solution. Extract of other plants contains different constituents with different functional groups, which act as reducing agents for reduction of silver ions to colloidal solution of nanoparticles. Extracts of leaf [46, 49], stem [50], fruit [51], bark [52], and root [53] have a potential for reduction of silver ions to colloidal solution of nanoparticles as cited by several workers. Furthermore, the above-mentioned results are evident for about a two-fold increase of the total phenolic content and flavonoids in *Tagetes erecta* aqueous leaf extract as compared to their derived colloidal solution of AgNPs. Phenolic content and flavonoids have been reported to be the most important phytochemicals responsible for the antioxidant capacity also. Plant-derived polyphenols display characteristic inhibition patterns toward the oxidative response. Thus, the higher amount of phenolic content and flavonoids in *Tagetes erecta* leaf extract can be taken as a fine indication of its higher antioxidant capability. Therefore, it may be concluded that the AgNPs biosynthesized from the leaf of *Tagetes erecta* possess high antioxidant activity which further suggest their therapeutic potential or can be used as a natural, renewable, and low-cost bioreducing agent. *Tagetes erecta* leaf extract could be used as an efficient green reducing agent for the production of AgNPs and can be explored for its applications in the prevention of free-radical-related diseases. On comparing with the earlier report in *Syzygium cumini* recorded higher total phenolic and flavonoid contents in plant extract than the AgNPs [54], *Asphodelus aestivus Brot.* extract can be used efficiently in the production of potential antioxidant AgNPs for commercial application [55].

3.4. Estimation of Antioxidant Activity in Aqueous Leaf Extract and AgNPs. Antioxidant activity of *Tagetes erecta* aqueous leaf extract and its derived AgNPs were assessed by DPPH scavenging assay and found the percentage of inhibition (PI) value 11.55 ± 0.12, 27.78 ± 1.34, and 38.56 ± 1.12 in aqueous leaf extract and 21.45 ± 0.44, 43.57 ± 0.87, and 62.58 ± 0.55 in AgNPs at different concentrations of the sample 200,400,600 μg/mL, respectively (Figure 3). The higher PI value was found in AgNPs as compared to aqueous leaf extract in all the three concentrations (μg/mL). Therefore, it may be concluded that the free-radical scavenging activity of aqueous leaf extract and AgNPs was directly related to their concentrations. On the other hand, we can say that the antioxidant activities are concentration-dependent and this trend was observed in both aqueous leaf extract and AgNPs.

DPPH is a stable and recognized synthetic solid radical for evaluating of antioxidant potential of compounds. The reducing power of compounds is directly proportional to their antioxidant activity. DPPH was reduced by accepting the hydrogen or electrons from silver nanoparticles, and this mechanism was quantified changing the color from purple to yellow by a spectrophotometer. This assay is frequently used in the measurement of the free-radical scavenging capacity of compounds present in medicinal plant extracts [56]. These results revealed that the AgNPs had greater free-radical scavenging potential than aqueous leaf extract of *Tagetes erecta*. Similar trends of enhanced DPPH scavenging activity by silver, platinum, and selenium, gold nanoparticles were observed by various workers [57, 58]. All in all, AgNPs may be useful for the development of newer and more potent antioxidants. The AgNPs having higher antioxidant properties could be used as treatment agents of many diseases caused by oxidative stress.

4. Conclusion

In the present study, the leaf extract of *Tagetes erecta* was used for the synthesis of silver nanoparticles. It was observed that the leaf extract contains multiple phytochemicals that play an important role (reducing as well as capping) during the synthesis of stable silver nanoparticles. Further, FTIR analysis confirms the role of phytochemicals during the synthesis of silver nanoparticles. *Tagetes erecta* leaf extract has a higher phenolic content and flavonoids, which indicates that it has a higher antioxidant capacity. As a result, AgNPs biosynthesized from *Tagetes erecta* leaf have a high antioxidant activity, implying that they have therapeutic potential or can be used as a natural, renewable, and low-cost bioreducing agent. *Tagetes erecta* leaf extract has the potential to be used as a green reducing agent for the production of AgNPs, as well as for the prevention of free-radical-related diseases. Moreover, significant antioxidant activity was observed for the plant-based nanoparticles and shows its potential application in the healthcare sector and could be used as treatment agents of many diseases caused by oxidative stress.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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