South African Journal of Chemical Engineering 26 (2018) 25–34

Contents lists available at ScienceDirect

South African Journal of Chemical Engineering

journal homepage: www.elsevier.com/locate/sajce

A Facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of *Ficus hispida* Linn. f. for catalytic, antioxidant and antibacterial applications

A.V. Ramesh[a], Dharmasoth Rama Devi[b], GangaRao Battu[b], K. Basavaiah[a,*]

[a] Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam, India
[b] A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, 530003, India

ARTICLE INFO

Keywords:
Green synthesis
Ag NPs
Ficus hispida Linn. f. leaf
Reduction of 4-Nitrophenol
Antioxidant activity
Antimicrobial activity

ABSTRACT

An aqueous leaf extract of *Ficus hispida* Linn. f. mediated silver nanoparticles (Ag NPs) have been prepared for reduction of 4-Nitrophenol, antioxidant and antibacterial activity against gram positive bacteria, *Bacillus subtilis* and gram negative bacteria, *Escherichia coli*. The rate of formation of Ag NPs is found to be depends on concentration of $AgNO_3$, concentration of aqueous leaf extract of *Ficus hispida* Linn. f., pH, temperature and reaction time. As prepared Ag NPs have been characterized by range of spectroscopic and microscopic techniquies such as UV–Vis spectroscopy, Fourier transform-infrared spectroscopy, powder X-ray diffraction, scanning electron microscopy, transmission electron microscopy. TEM images clearly showed that the formation of spherical shape Ag NPs with an average particle size of 20 nm. As prepared Ag NPs showed high catalytic activity towards the reduction of 4-Nitrophenol to 4-Aminophenol. Green synthesized Ag NPs showed enhanced antioxidant activity and also showed potent antibacterial activity against of both *E. coli* and *Bacillus subtilis*.

1. Introduction

During the past decade, the synthesis of nanomaterials have received a great attention for technological application due to their unique physical and chemical properties (Sharma et al., 2009). Among metal nanomaterials, silver nanoparticles (Ag NPs) have attracted attention of researchers owing to their versatile applications, such as antioxidant (Kalaiyarasan et al., 2017), anticancer (Nayak et al., 2015), antibacterial (Ghaedi et al., 2015), catalysis and pollution abatement (Sun et al., 2018). Ag NPs are biocompatible to human cells at lower concentrations and inhibits the growth of microorganisms (Sharma et al., 2009). Therefore Ag NPs have been used extensively as antimicrobial agents in cosmetics (Kokura et al., 2010), textile coatings (Vigneshwaran et al., 2007; Durán et al., 2007) and food industry (Chaudhry and Castle, 2011). Numerous methods have been reported for synthesis of Ag NPs including physical and chemical methods such as thermal decomposition (Navaladian et al., 2006), microwave assisted process (Sreeram et al., 2008), chemical reduction (Yu, 2007), and electrochemical reduction (Starowicz et al., 2006). In all these methods, reducing agents such as Tollen's reagent, sodium borohydride [$NaBH_4$] and N, N- dimethyl formamide (DMF) were used for reduction of silver ions (Ag+) into Ag NPs. Moreover inorder to prepare aggregation free, controlled size and shape of Ag NPs, the toxic surfactants or capping agents such as tri-sodium citrate, dodecyl sulfonic acid etc. were used (Sharma et al., 2009). Therefore it is highly desirable to synthesize Ag NPs via non-hazardous, biocompatible, eco-friendly and sustainable methods for biomedical applications. Even though many biological green methods have been reported for synthesis of Ag NPs, the plant mediated synthesis emerged as a potential method due to non toxic, cost-effective, rapid and their biomedical application. Furthermore, in microorganisms assisted green synthesis of Ag Nps, it is very difficult to maintain the microbial culture (Kumar et al., 2011, Saha et al., 2017, Otari et al., 2012, Jha et al., 2009.) (Table S2).

Many reports are available on the green synthesis of Ag NPs using numerous plant extracts such as fruit extract of *Gmelina arborea* (Saha et al., 2017), aqueous leaf extract of *Azadirachta indica* (Ahmed et al., 2016), extract of *Parkia speciosa* Hassk pods (Fatimah, 2016), banana peel extract (Ibrahim, 2015), leaf extract of *Chenopodium murale* (Abdel-Aziz et al., 2014), leaf extract of *Cinnamomum camphora* (Huang et al., 2007), leaf extract of *Azadiracta indica* (Shankar et al., 2004), *Capsicum annuum* L. (Li et al., 2007) *Aloe vera* (Chandran et al., 2006), *Embilica officinalis* (Ankamwar et al., 2005), *Camellia sinensis* extract (Vilchis-Nestor et al., 2008), *Acalypha indica* leaf extracts (Krishnaraj et al., 2010), latex of *Jatropha curcas* (Bar et al., 2009) and natural rubber

* Corresponding author.
E-mail address: klbasu@gmail.com (K. Basavaiah).

https://doi.org/10.1016/j.sajce.2018.07.001
Received 13 November 2017; Received in revised form 3 June 2018; Accepted 9 July 2018

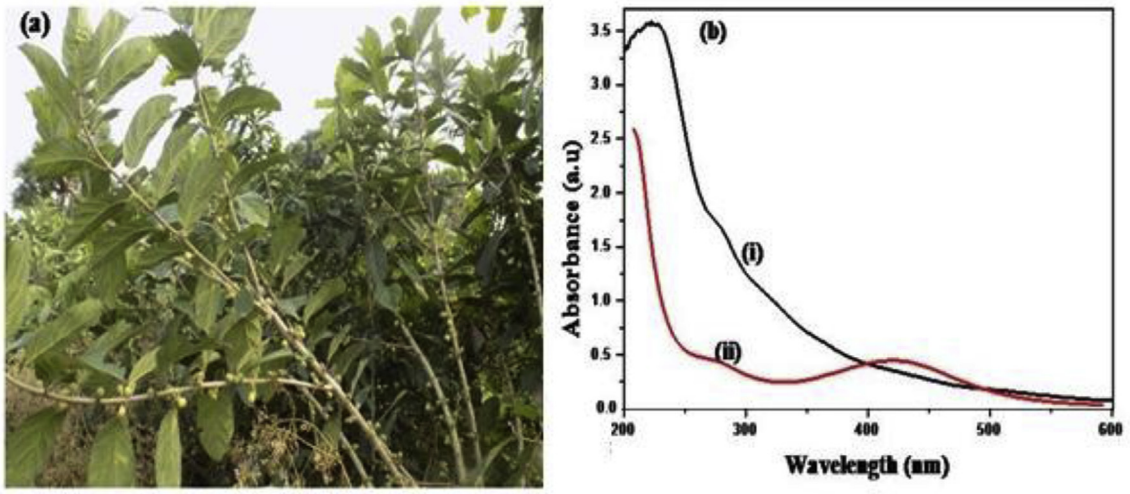

Fig. 1. (a) Image of fresh leaves of *Ficus hispida* Linn. f. (b) UV–Vis spectra of (i) aqueous leaf extract of *Ficus hispida* Linn. f. and (ii) synthesized Ag NPs.

(Abu Bakar et al., 2007). To the best of my knowledge, there were no reports on preparation of Ag NPs using an aqueous leaf extract of *Ficuss hispida* Linn. f. Aqueous leaf extract of *Ficus hispida* Linn.f. contains wide varieties of phytochemical compounds (metabolites) like sterols, triterpenoic acid, flavonoids, phenols and tannins (Table S1). Phenols, flavonoids and triterpenoic acid contain active oxygen that have potential to donate electrons for reduction of silver precursor into Ag NPs. Triterpenoic acid present in plant extract has $–COO^-$ group acts as chelating group for capping of the synthesized Ag NPs. Thus the phytoconstituents present in the aqueous leaf extract of *Ficus hispida* Linn. f. act as reducing and capping agent for the formation of Ag NPs (Ashish et al., 2014).

In this study, we have synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn.f. The formed Ag NPs were characterized with spectroscopic and microscopic techniques. The catalytic activity of synthesized Ag NPs have been investigated by performing a model reaction, the reduction of 4-Nitrophenol to 4-Aminophenol in presence of $NaBH_4$. The free radical scavenging activity of Ag NPs against DPPH radical and antibacterial activity of Ag NPs against gram positive and gram negative bacteria were also investigated.

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$), Sodium borohydride ($NaBH_4$), Sodium hydroxide (NaOH), Orthophosphoric acid (H_3PO_4), 4-Nitrophenol, DPPH (1, 1 -diphenyl-2-picrylhydrazyl), were purchased from Merck, India and used without further purification. Source of *E. coli,* and *B. subtilis* strains collected from IMTECH, Chandigarh, India. The leaves of *Ficus hispida* Linn. f. were collected from forest of Chinthapalli, Andhra Pradesh, India during May 2015. Botanical aspect of collected plant materials was identified and authenticated by plant taxonomist Professor S. B. Padal, Andhra University, Visakhapatnam, A.P, India. Milli-Q water was used throughout the synthesis process.

2.2. Preparation of plant extract

The fresh leaves of *Ficus hispida* Linn. f. were washed several times with running tap water and followed by Milli-Q water to remove the dust and then the leaves were dried under shade for 15 days. The dried leaves were powdered using blender. The 5 (W/V) % extract was prepared by dissolving 5 g of leaf powder in 100 mL Milli-Q water and the solution was heated for 20 min at 60 °C. Then the aqueous leaf extract was filtered through the Whatman NO.1qualitative cellulose filter paper with diameter 125 mm and pore size: 11 μm and the filtrate was stored at 4 °C for further use.

2.3. Synthesis of Ag NPs

In a typical procedure, 10 mL of 5 (W/V) % aqueous leaf extract of *Ficus hispida* Linn. f. was added to 100 mL of 4 mM $AgNO_3$ aqueous solution in a 250 mL Erlenmeyer flask. Then the reaction mixture was stirred on a water bath for 60 min at 90 °C. The pH of the reaction mixture was adjusted to 9 by using 0.1 N NaOH and 0.1 N H_3PO_4. The colour of the reaction mixture was turned to reddish brown, which indicates the formation of Ag NPs. The same procedure was adopted for synthesis of Ag NPs at different concentrations of $AgNO_3$, plant extract, pH and temperature.

2.4. Characterization

The UV–Visible spectra (UV–Vis) of as prepared Ag NPs were recorded using a Shimadzu (2450 – SHIMADZU) spectrophotometer. Fourier transform-infrared (FTIR) spectra were recorded in the range of 4000-400 cm^{-1} with a SHIMADZU-IR PRESTIGE-2 Spectrophotometer. Powder samples were mixed thoroughly with potassium bromide (KBr) and pressed into thin pellets. The powder X-ray diffraction (XRD) patterns were obtained by PANalytical X'pert pro diffractometer at 0.02°/sec scan rate with Cu-kα_1 radiation (1.5406 A^0, 45 kV, 40 mA). FE-SEM (Field emission-Scanning electron microscopy) images were acquired (FE-SEM model JEOL JSM-7600F FEG-SEM) at accelerating voltages of 0.1–30 kV. TEM (Transmission electron microscopy) images were obtained (TEM model FEI Technai 20 U Twin) at an accelerating voltages of 120 and 200 kV.

2.5. Reduction of 4-Nitrophenol to 4-Aminophenol

The reduction of 4-Nitrophenol to 4-Aminophenol by using aqueous leaf extract of *Ficus hispida* Linn. f. as control without Ag NPs in presence of $NaBH_4$ was initially investigated. Then, the catalytic activity of as-prepared Ag NPs was investigated by performing a reduction of 4-Nitrophenol to 4- Aminophenol in the presence of $NaBH_4$. During the catalytic reduction, the absorption peak of 0.1 mL of 1 mM 4-Nitrophenol which was taken in standard quartz cuvette (path length 1 cm) is formed at 318 nm with light yellow colour. To this 4–Nitrophenol, freshly prepared 0.2 mL of 0.2 M $NaBH_4$ is added then intense yellow colour is formed, this indicates the formation of 4-

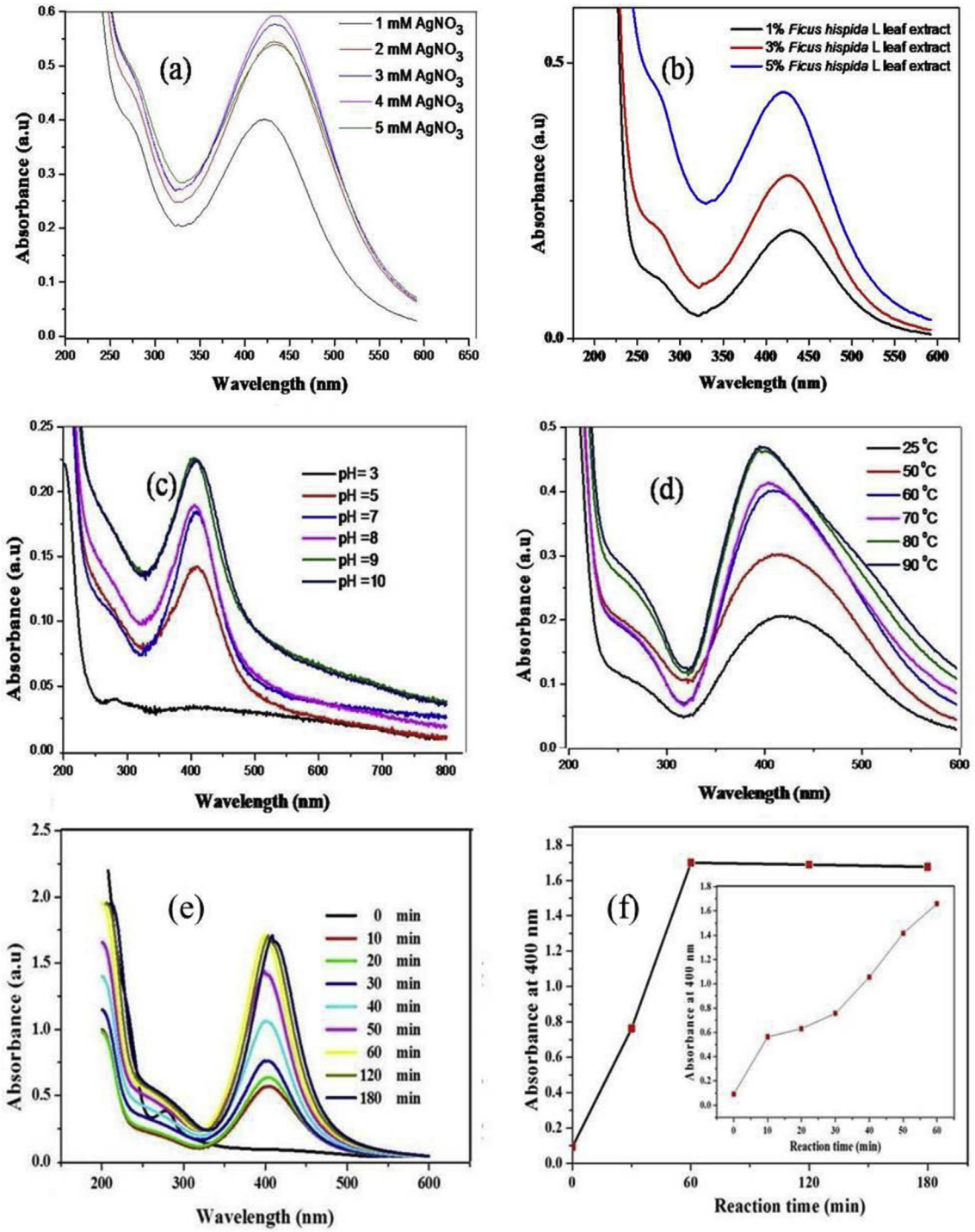

Fig. 2. UV–Vis spectra of Ag NPs prepared by at different (a) concentration of $AgNO_3$, (b) concentration of aqueous leaf extract of *Ficus hispida* Linn. f., (c) pH (d) temperature (e) reaction time and (f) kinetics of reaction time.

Nitrophenolate ion. The absorption peak of 4-Nitrophenolate ion is formed at 400 nm. To this mixture, 10 mg Ag NPs was added and diluted up to 3 mL using Milli-Q water. The reaction was monitored using UV–Vis spectrophotometer by observing the change in absorbance intensity of 4-Nitrophenolate ion at 400 nm. The catalytic reduction of 4-Nitrophenol in presence of $NaBH_4$ at different amount of Ag NPs (5 mg, 10 mg, and 15 mg) was investigated.

2.6. Determination of free radical scavenging activity by DPPH assay

2.6.1. Principle

The DPPH (1, 1 -diphenyl-2-picrylhydrazyl) assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non-radical form of yellow coloured diphenyl-picryl hydrazine. Using a DPPH method, the free radical scavenging activity of Ag NPs was investigated according to the procedure described by Clarke (Clarke et al., 2013)). To aliquot of 3 ml of 0.004 % DPPH solution in ethanol, 0.1 mL of Ag NPs at different concentrations (50, 100, 200 and 250 µg/mL) were

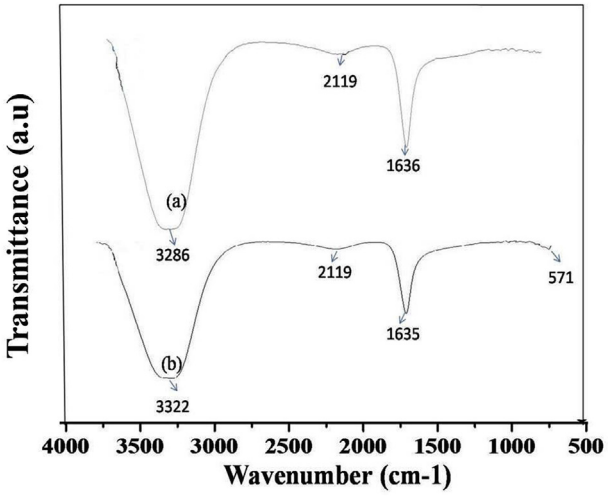

Fig. 3. FTIR spectra of (a) *Ficus hispida* Linn. f. leaf extract and (b) Ag NPs.

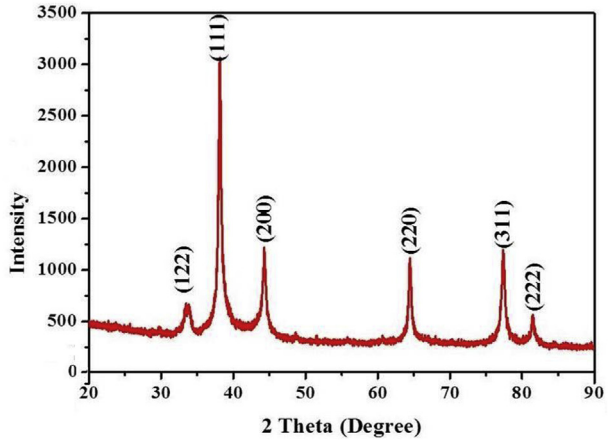

Fig. 4. Powder X-Ray diffraction pattern of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn. f.

added. Each mixture was left for 20 min and the absorbance was measured at 517 nm. DPPH scavenging activity was calculated using equation (1).

$$\text{Scavenging activity}(\%) = \frac{[(A_c - A_s)]}{A_c} \times 100 \quad (1)$$

Where "A_c" is absorbance of control without Ag NPs and "A_s" is the absorbance in the presence of the Ag NPs.

2.7. Investigation of antimicrobial activity

2.7.1. Media preparation

The Mueller Hinton agar medium was used for investigation of antibacterial activity. It was prepared by dissolving synthetic Mueller Hinton agar powder in distilled water. The pH of the medium was adjusted to 7 with 1 N NaOH. The medium and petri plates (100 mm × 15 mm) were autoclaved at 121 °C, 15 lbs for 20 min. The autoclaved medium was allowed to cool to 45 °C and transferred into petri plates (20 mL/plate) under disinfected conditions of laminar air flow.

2.7.2. Antibacterial activity

The antibacterial activity of synthesized Ag NPs by the aqueous leaf extract of *Ficus hispida* Linn. f. was investigated against various pathogenic bacteria such as *B. subtilis* MTCC211 and *E. coli* MTCC443 *via* the Agar well diffusion method. The diameter of inhibition zones (mm) around each well with Ag NPs is represented in Fig. 9 and Table 3. In Fig. 9 (a), (b) (c) and (d); well No 1, 2, 3, 4, 5, C and P represents 25 μg/mL, 50 μg/mL, 100 μg/mL Ag NPs, 25 μg/mL Streptomycin (positive control), combination of 25 μg/mL Streptomycin (positive control) + 25 μg/mL Ag NPs, 3% DMSO (Dimethyl sulfoxide) as negative control and aqueous leaf extract of *Ficus hispida* Linn. f. without Ag NPs including 3 % DMSO negative control respectively. Diffusion of compounds, antibiotics were allowed at room temperature for 1 h. All of the plates were then covered with lids and incubated at 37 °C for 24 h. After incubation, plates were observed for the zone of bacterial growth inhibition. The size of inhibition zones was measured and antibacterial activity of the compounds was expressed in terms of the average diameter of inhibition zone in millimeters. Each Ag NPs concentration was tested in triplicate with two independent experiments and the mean values of inhibition zone diameters were taken.

3. Results and discussion

Ag NPs were prepared by using an aqueous mixture of 4 mM $AgNO_3$ and aqueous leaf extract of *Ficus hispida* Linn. f. at a 10:1 M ratio in presence of alkaline medium. The plant *Ficus hispida* Linn.f. belongs to the Moraceae family and all parts of this plant are used to be antidysenteric and to have activity against jaundice, antidiabetic activity, hypoglycemic activity and hemorrhage (Nadkarni and Nadkarni, 1955; Rastogi et al., 2001; Peraza-Sánchez et al., 2002). The phytochemicals present in the aqueous leaf extract of *Ficus hispida* Linn. f. are responsible for the formation of Ag NPs. The colour of the reaction mixture of $AgNO_3$ and aqueous leaf extract of *Ficus hispida* Linn. f. turned to reddish brown, which indicates that the formation of Ag NPs. The formation of Ag NPs was further confirmed by range spectroscopic and microscopic techniques.

3.1. Absorption spectroscopy

The UV–Vis absorption spectra of synthesized Ag NPs and aqueous leaf extract of *Ficus hispida* Linn. f. were depicted in Fig. 1 (b). The characteristic absorption peak at 423 nm indicates the formation of Ag NPs which is primarily due to the surface plasmon resonance (SPR) absorption in Ag NPs (Mulvaney, 1996). Inorder to find out best synthesis condition of Ag NPs, the optimization were carried at different experimental parameters such as concentration of $AgNO_3$, concentration of aqueous leaf extract of *Ficus hispida* Linn. f., temperature, pH and reaction time.

UV–Visible spectra of Ag NPs prepared at different concentration of $AgNO_3$ were presented in Fig. 2 (a). As shown in Fig. 2 (a), the intensity of the SPR absorption peak was increased (424 nm- 437 nm) with increase of concentration of $AgNO_3$ (1 mM–3 mM), the peak was red shifted (424–437 nm). However, the SPR peak was blue shifted (437 nm–433 nm) for Ag NPs prepared at 4 mM of $AgNO_3$.

UV–Vis spectra of Ag NPs prepared at different concentration of aqueous leaf extract of *Ficus hispida* Linn. f. (1%, 3%, and 5%) were presented in Fig. 2 (b). As the concentration of aqueous leaf extract increases, the intensity of the SPR peak of Ag NPs also increases. The increase in intensity of SPR absorption peak can be ascribed to the presence of more number of biomolecules in aqueous leaf extract of *Ficus hispida* Linn. f. which are responsible for the reduction of Ag^+ to Ag^0. The SPR peak was blue shifted (430 nm–423 nm) which indicated that the formation of smaller size Ag NPs (Smitha et al., 2008).

UV–Vis spectra of Ag NPs obtained at different pH were depicted in Fig. 2 (c). It can be seen that the intensity of SPR peak increases with increase of pH from 3 to 9. The rate of the formation of Ag NPs is more at pH = 9. Fig. 2 (c) clearly showed that the alkaline environment improved the reducing and stabilizing potential of aqueous leaf extract of *Ficus hispida* Linn. f. towards the Ag NPs formation. The number of formation of the Ag NPs increased with alkaline pH due to the

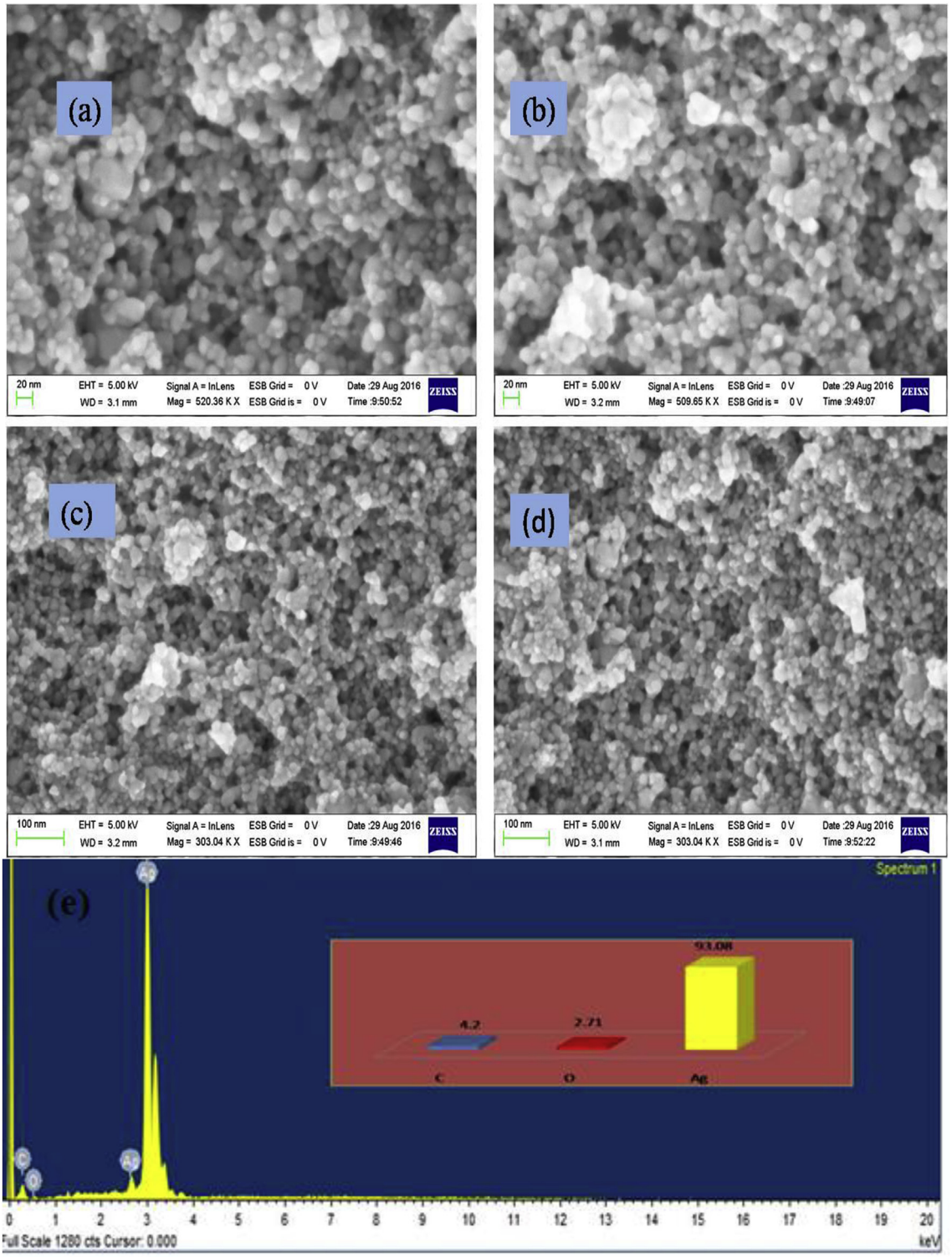

Fig. 5. (a–d) FE-SEM images of synthesized Ag NPs from aqueous leaf extract of *Ficus hispida* Linn. f. at different magnification and (e) EDX spectrum of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn. f.(inset the weight (%) composition).

enhancing reactivity of the aqueous leaf extract of *Ficus hispida* Linn. f. and thus absorption peaks of the formed Ag NPs undergo blue-shift with smaller particle size. However, at a pH value more than 9, an agglomeration of Ag NPs was observed.

The effect of temperature on the formation of Ag NPs was presented in Fig. 2 (d). The intensity of SPR peak was increased with increase of temperature. As the reaction temperature increases, leads to a quick reduction of the Ag^+ ions and hence successive homogeneous nucleation of Ag NPs with smaller particle size. As the temperature increases from 35 °C to 90 °C, the intensity of SPR peak is also increased with blue shifted (426 nm–399 nm). Further increase of temperature above 90 °C, the intensity of SPR peak decreases and hence the optimum temperature for the synthesis of Ag NPs is 90 °C.

The kinetics of Ag^+ reduction were obtained by monitoring the intensity of the SPR peak as a function of time (Fig. 2(e) and (f)). The SPR peak appears immediately after the addition of the 10 mL of 5 %

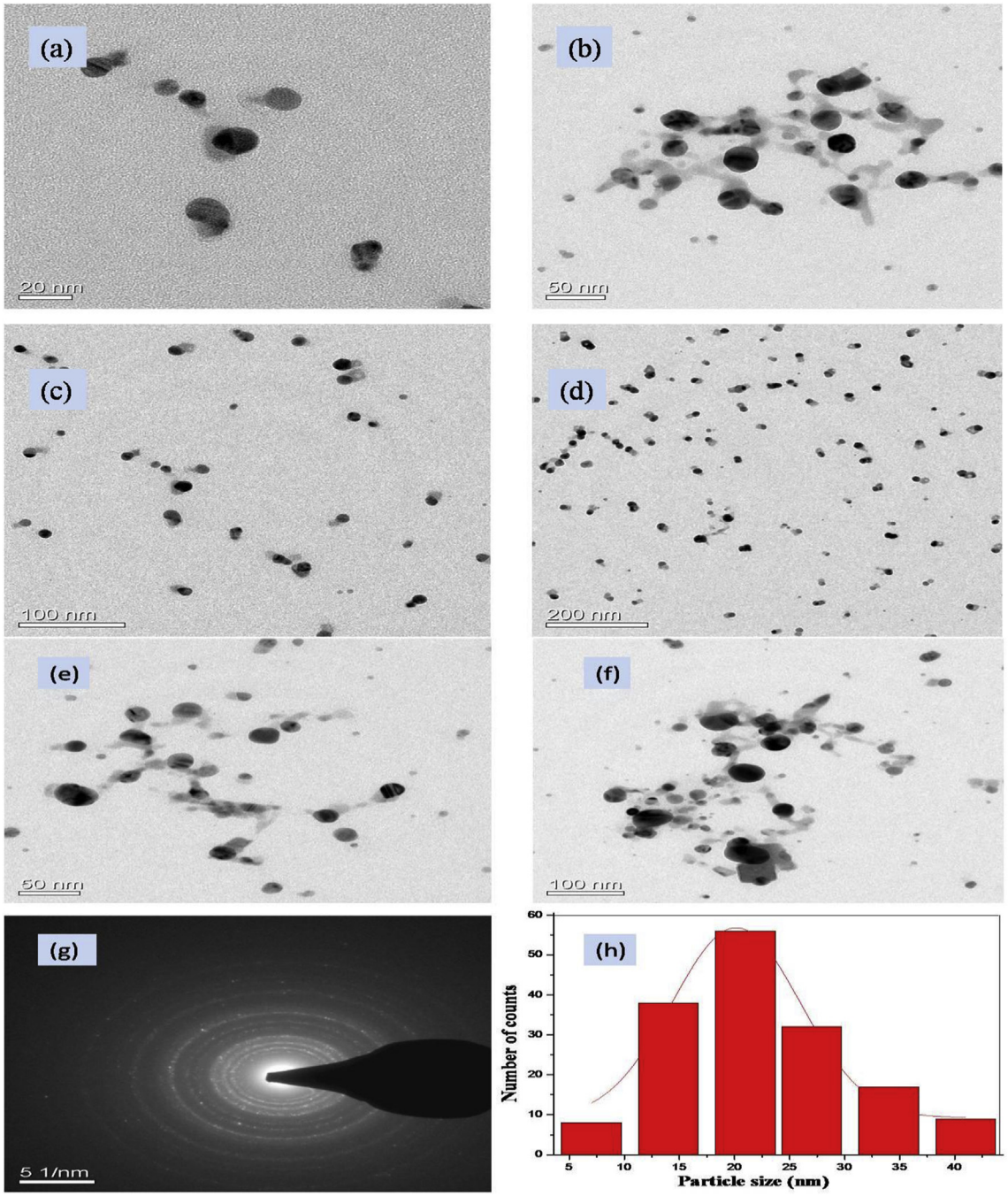

Fig. 6. (a-f) TEM images of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn. f.and (g) selected area electron diffraction pattern and (h) particle size distribution histogram.

aqueous leaf extract of *Ficus hispida* Linn. f. to the 100 mL of 4 mM $AgNO_3$ and increases intensity as a function of time with no significant peak shift (Fig. 2(e) and (f)). This shows the bioreduction of Ag^+ ions and thus increases in the number of Ag NPs. As shown in Fig. 2 (e) and 2 (f), the increase in intensity of SPR peak saturates within 60 min. Any incubation time beyond 60 min shows almost no further increase in SPR intensity suggesting that the completion of the reaction is within 60 min.

3.2. FTIR

FTIR spectra of aqueous leaf extract of *Ficus hispida* Linn. f. and Ag NPs were recorded to identify the interaction of functional groups of aqueous leaf extract of *Ficus hispida* Linn. f. responsible for reduction of $AgNO_3$ and the capping of subsequently formed Ag NPs. As shown in Fig. 3 both FTIR spectra of an aqueous leaf extract of *Ficus hispida* Linn. f. and Ag NPs obtained from aqueous leaf extract of *Ficus hispida* Linn. f. have close similarities with some marginal shifts in peak position, clearly indicate the presence of the residual plant extract on Ag NPs. The FTIR spectrum of the aqueous leaf extract of *Ficus hispida* Linn. f. exhibited characteristic stretching frequencies at 3286, 2119 and 1636 cm^{-1}. The characteristic band at 3286 and 1636 cm^{-1} are ascribed to the presence of an O-H group of phenolic compound/triterpenoic acid, C=O of triterpenoic acid group. In the case of Ag NPs synthesized using aqueous leaf extract of *Ficus hispida* Linn. f., there was a shift in the absorbance peak of O-H group of phenolic compound or

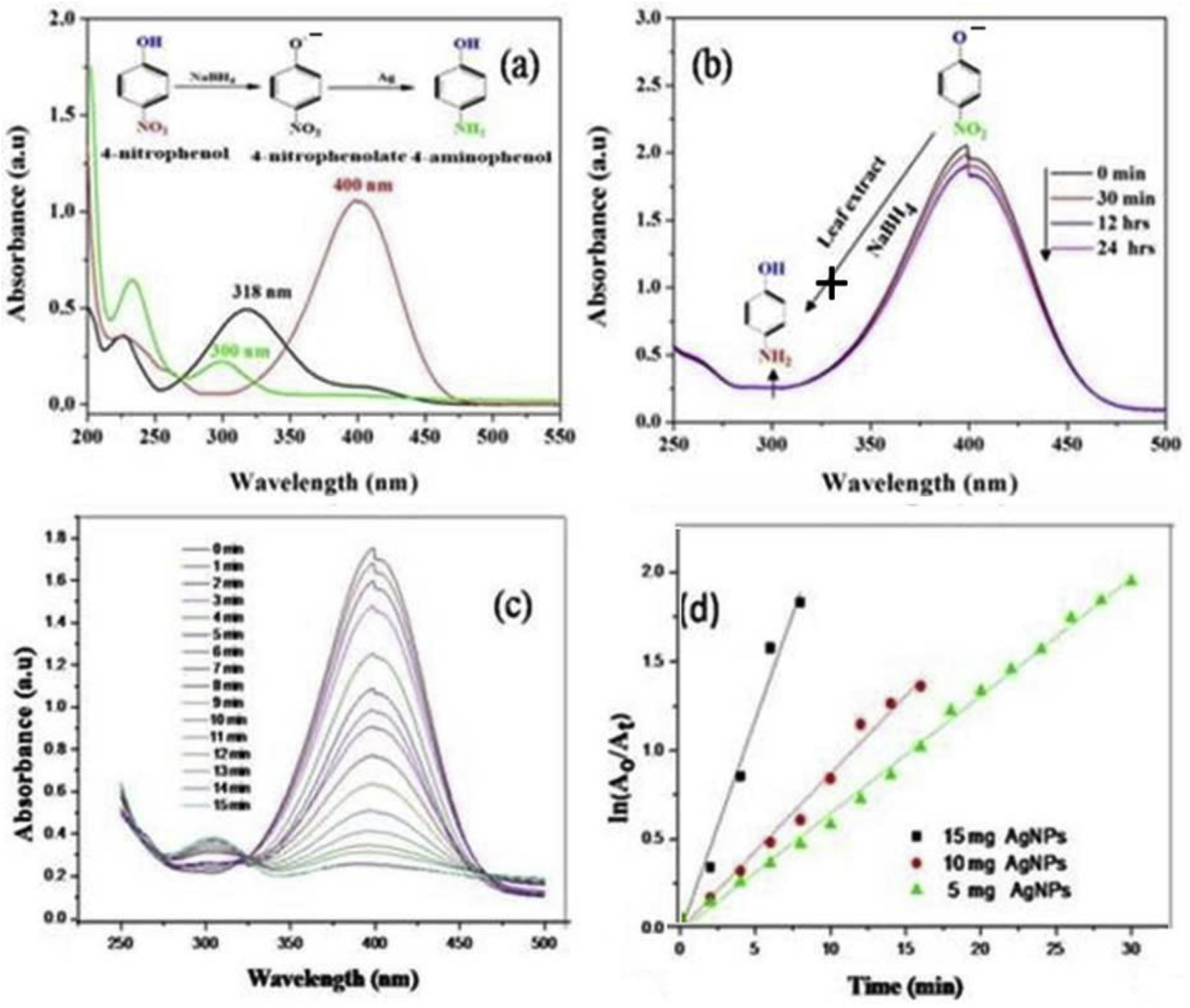

Fig. 7. (a) Formation of 4-Aminophenol from 4- Nitrophenol by Ag NPs in the presence of $NaBH_4$, (b) Reduction of 4-Nitrophenol using plant extract in presence of $NaBH_4$, (c) Reduction of 4-Nitrophenol using Ag NPs in presence of $NaBH_4$ and (d) kinetics of reduction reactions.

triterpenoic acid from 3286 to 3322 cm^{-1} implying that the reduction of silver ions with hydroxyl groups and capping of synthesized Ag NPs with the carboxyl group of the extract as is evident. Furthermore, one additional peak at 571 cm^{-1} was observed for in the case of Ag NPs which is ascribed to the symmetric and asymmetric vibration of Ag-O, where oxygen comes from the hydroxyl functional group of plant extract. The phytochemical analysis of aqueous leaf extract of *Ficus hispida* Linn. f. based on FTIR spectrum strongly suggested that the presence of triterpenoic acid, flavonoids and polyphenols, apart from other phytochemicals, which were mainly responsible for the preparation of the Ag NPs by reducing the $AgNO_3$ (Kumar et al., 2016).

3.3. XRD

The crystalline nature of Ag NPs was confirmed using powder XRD. The XRD pattern of Ag NPs synthesized using an aqueous leaf extract of *Ficus hispida* Linn. f. was shown in Fig. 4. The Bragg reflection peaks were observed at 2θ values of 33.44 °, 38.14 °, 44.31 °, 64.45 °, 77.37 °, 81.48 ° which could be indexed to (122), (111), (200), (220), (311) and (222) planes of the fcc structure of pure silver NPs (JCPDS, File No. 04–0783). The intense reflection of (111) plane in contrast to the other planes may specify the growth direction of the nano crystals. The average crystallite sizes of the Ag NPs were estimated by the Scherrer equation (Eq. (2))

$$D = k\lambda/\beta \cos\theta \quad (2)$$

Where "D" is the particle diameter size, "k" is a constant equals 1, " λ" is wavelength of X-ray source (0.1541 nm), "β" is the full width at half maxima (FWHM) and θ is the diffraction angle corresponding to the lattice plane. The average crystallite size of Ag NPs according to Scherrer equation with the width of (111) plane is found to be 30.5 nm.

3.4. Morphology

Surface morphology of synthesized Ag NPs was investigated by FESEM and TEM. Representative FESEM images of synthesized Ag NPs using an aqueous leaf extract of *Ficus hispida* Linn. f. were depicted in Fig. 5. FE-SEM results clearly show spherical shape Ag NPs. The presence of elemental silver (Ag), Carbon (C) and Oxygen (O) confirms not only the successful formation of Ag NPs, but also capping of Ag NPs by phytoconstituents present in plant extract. TEM images of as prepared Ag NPs using an aqueous leaf extract of *Ficus hispida* Linn. f. were presented in Fig. 6. TEM results revealed the Ag NPs was spherical morphology with average particle size of 20 nm. The selected area electron diffraction (SAED) patterns (Fig. 6. (g)) shows the ring like diffraction pattern indicated that polycrystalline nature of Ag NPs and

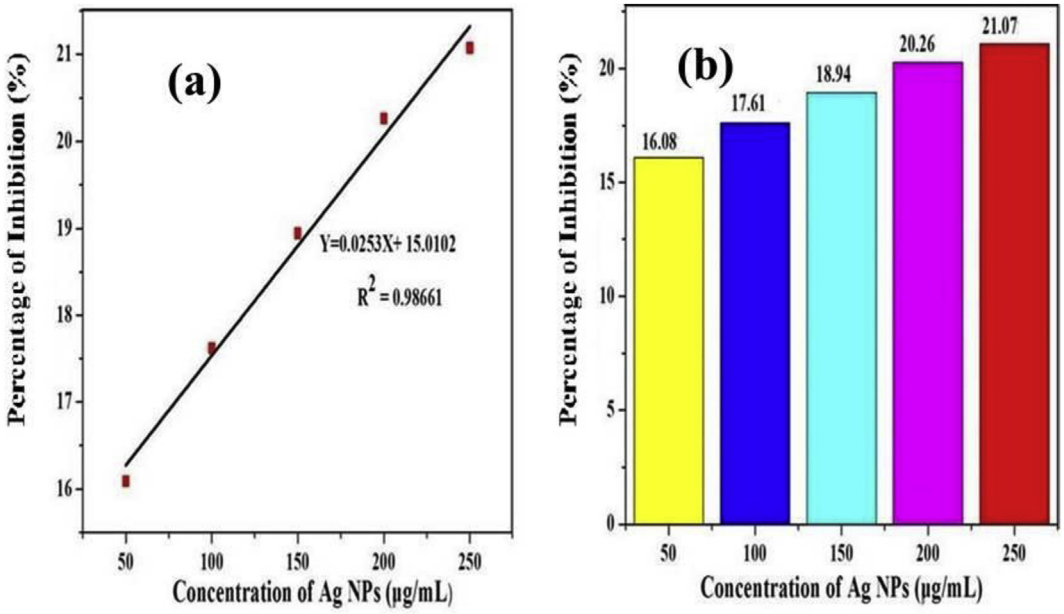

Fig. 8. (a),(b) The antioxidant efficacy of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn. f. against DPPH

is in very good agreement with previous reported literature (Kumar et al., 2016).

4. Reduction of 4-Nitrophenol to 4-Aminophenol using Ag NPs

The catalytic activity of synthesized Ag NPs by aqueous leaf extract of *Ficus hispida* Linn. f. was investigated by the reduction of 4-Nitrophenol to 4-Aminophenol in the presence of excess $NaBH_4$. This reduction reaction takes place via formation of an intermediate 4-Nitrophenolate ion (Fig. 7 (a)). Usually, the concentration of BH_4^{-1} is very high compared with that of 4-Nitrophenol during the catalytic reduction reaction, so this reaction is pseudo first ordered reaction with respect to 4 - Nitrophenol. The reduction of 4-Nitrophenol to 4- Aminophenol by using aqueous leaf extract of *Ficus hispida* Linn. f. as control without Ag NPs in presence of $NaBH_4$ was initially investigated for 24 h and found that 4-Nitrophenol was not reduced into 4-Aminophenol in the presence of excess $NaBH_4$ (Fig. 7(b)). Then as prepared Ag NPs was employed as catalyst for reduction of 4- Nitrophenol to 4-Aminophenol in the presence of $NaBH_4$. When 10 mg of Ag NPs was introduced to the reaction mixture, the intensity of absorption peak at 400 nm was decreased and a new peak at 300 nm was observed and its intensity increases with time (Fig. 7 (c)), which indicates that the reduction of 4-Nitrophenol to 4-Aminophenol occurs. After the completion of the reaction, the Ag NPs catalyst was collected from the reaction system by centrifugation and washed successively with deionized water and dried for use in the next cycle. As shown in Fig. 7 (d), the kinetics of the reduction as a function of catalyst dose was obtained a linear plot from the pseudo first order kinetic mode ($\ln (A_0/A_t)$ versus time (t)). The rate constant (k) of different doses of Ag NPs (Table 1) was determined from the slope of Fig. 7 (d). The increased rate constants with an increase in Ag NPs concentration is ascribed to an increase in the number of active reaction sites for the reduction of 4-Nitrophenol (Saif et al., 2016).

5. Antioxidant activity

The antioxidant activity of Ag NPs was investigated by DPPH assay method. Ag NPs was used as a radical scavenger and DPPH was used as the radical source. The colour of DPPH solution was changed from deep violet to pale yellow in the presence of Ag NPs. The absorbance of DPPH is at 517 nm is gradually decreases with increases of concentrations of Ag NPs, which is further confirmed the free radical scavenging activity of Ag NPs (Fig. 8 (a)). This result shows that the antioxidant efficiency of Ag NPs is dose dependent (Table 2). The all values represented in the tables are average ± SD of results of three separately conducted experiments. Fig. 8(b) shows the Ag NPs show free radical scavenging activity up to 21.07 ± 0.02% in 250 µg/mL concentration of Ag NPs (Abdel-Aziz et al., 2014).

6. Antibacterial activity

The antibacterial activity of aqueous leaf extract of *Ficus hispida* Linn. f. was investigated using the 3 % DMSO as a negative control. The negative control and leaf extract did not show antibacterial activity (Fig. 9 (b), 9(d)). Antibacterial activity of Ag NPs was found to be dose-dependent. As the concentration of Ag NPs increases, antibacterial activity of Ag NPs also increases due to increase in the interaction of Ag NPs with sulphur containing proteins of bacteria (Feng et al., 2000), resulting in cell death. Hence, comparing concentrations of Ag NPs represented in (Table 3), at 100 µg/mL concentration, Ag NPs were found to be have antibacterial activity against gram negative bacteria *E. coli* (14 mm) and gram positive bacteria *B. subtilis* (12 mm) respectively (Fig. 9 (e)) (Ibrahim, 2015). The interaction of bacteria and Ag NPs can be generally described through three approaches. The first approach is for the duration of their interactions can be the electrostatic attraction between positively charged membrane proteins present on the surface of bacteria and negatively charged Ag NPs (carboxylate stabilized). The second approach is the physicochemical alterations in the bacterial cell wall which can cause expulsion of intracellular material and may possibly causes cell death. The third approach is the tendency of Ag NPs to penetrate through bacterial cell membrane. As a result, Ag NPs may prevent DNA replication and respiration of bacteria and may cause bacterial cell death (Agnihotri et al., 2014).

7. Conclusion

In conclusion, the Ag NPs were successfully synthesized using aqueous leaf extract of *Ficus hispida* Linn. f. via green route method. The phytochemical present in aqueous leaf extract of *Ficus hispida* Linn. f. can act as reducing and capping agent for the formation of Ag NPs. The synthesized Ag NPs were characterized with UV–Vis, FT-IR, SEM, EDX, and TEM analysis authenticate the formation of Ag NPs. The spherical

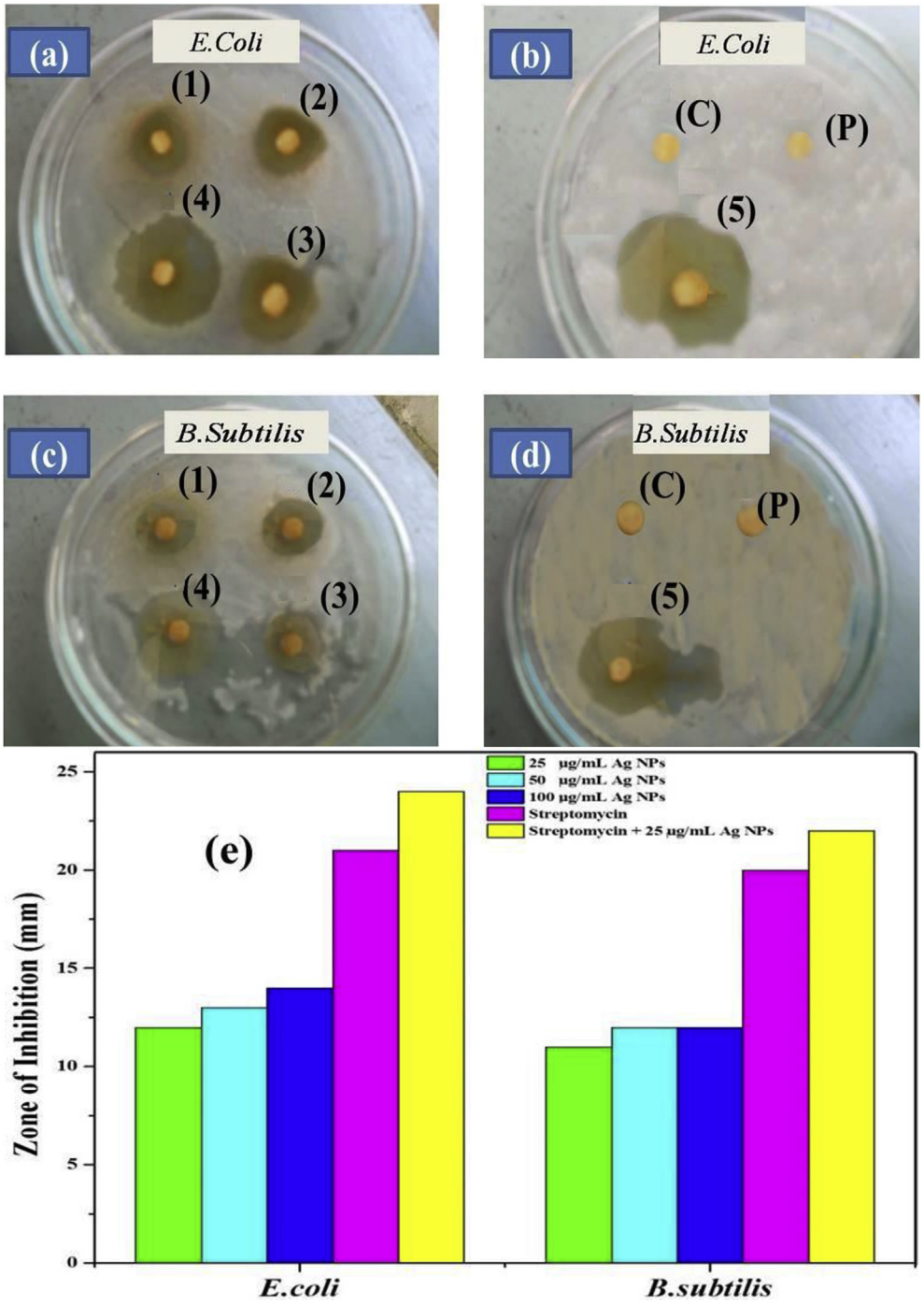

Fig. 9. (a,b) Antibacterial activity of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn. f. against gram negative bacteria *E. coli*; (c,d) gram positive bacteria *B. subtilis* and (e) Zone of inhibition of Ag NPs against *E. coli and B. subtilis* microorganisms.

Table 1
The kinetic parameters obtained from the reduction of 4 - Nitrophenol to 4 -Aminophenol using synthesized Ag NPs from aqueous leaf extract of *Ficus hispida* Linn.f.

Sample number	Concentration of Ag NPs (mg)	Calculated rate constant (min^{-1})	R^2 value	Time for completion of reduction of 4-Nitrophenol (min)
1.	5	0.0595	0.9879	30
2.	10	0.0921	0.9793	15
3.	15	0.1652	0.9742	08

Table 2
The antioxidant efficacy of different concentrations of synthesized Ag NPs using aqueous leaf extract of *Ficus hispida* Linn.f. against DPPH·.

Sample number	Concentration of Ag NPs (µg/mL)	% of DPPH scavenging effect (mean ± S.D)
1.	50	16.08 ± 0.01
2	100	17.61 ± 0.02
3.	150	18.94 ± 0.04
4.	200	20.26 ± 0.03
5.	250	21.07 ± 0.02

Table 3
Zone of inhibition values obtained from the Antibacterial activity of Ag NPs synthesized using aqueous leaf extract of *Ficus hispida* Linn.f. against both gram-negative and gram-positive bacteria strains.

Name of the bacteria	Zone of Inhibition (mm)							
	DMSO Negative Control	Plant leaf extract	25 µg/mL of AgNPs	50 µg/mL of Ag NPs	100 µg/mL Of Ag NPs	25 µg/mL Streptomycin (Positive control)	25 µg/mL Streptomycin +25 µg/mL of Ag NPs	% of Enhancement
Escherichia coli (gram negative)	0	0	12	13	14	21	24	1.03
Bacillus subtilis (gram positive)	0	0	11	12	12	20	22	1.02

morphology was observed for Ag NPs with average particle size of 20 nm. The synthesized Ag NPs have been used as an efficient catalyst for the reduction of 4-Nitrophenol to 4-Aminophenol. Furthermore, the obtained Ag NPs were act as antioxidant and posses potent antibacterial activity against both *E. coli* and *B. subtilis*.

Acknowledgement

The author is grateful to UGC-SERO, Hyderabad for awarding teacher fellowship under FDP, XII plan of UGC, New Delhi. Author also acknowledge to UGC-SAP-DRS-I (No.F.540/18/DRS-I/2016), Department of Inorganic and Analytical chemistry, Andhra University and also DST-FIST (5R/FIST/CSI-241/2012(C)), Department of Inorganic and Analytical chemistry, for their financial assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.sajce.2018.07.001.

References

Abdel-Aziz, M.S., Shaheen, M.S., El-Nekeety, A.A., Abdel-Wahhab, M.A., 2014. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract. J. Saudi Chem. Soc. 18, 356–363.
Abu Bakar, N.H.H., Ismail, J., Abu Bakar, M., 2007. Synthesis and characterization of silver nanoparticles in natural rubber. Mater. Chem. Phys. 104, 276–283.
Agnihotri, S., Mukherji, S., Mukherji, S., 2014. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv. 4, 3974–3983.
Ahmed, S., Saifullah, Ahmad, M., Swami, B.L., Ikram, S., 2016. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J. Radiat. Res. Appl. Sci. 9, 1–7.
Ankamwar, B., Damle, C., Ahmad, A., Sastry, M., 2005. Biosynthesis of gold and using emblica officinalis fruit extract, their phase transfer and transmetallation in an organic solution. J. Nanosci. Nanotechnol. 5, 1665–1671.
Ashish, J., Sanjeeb Kumar, M., Vanaja, N., Abhinav, R., Naisarg, M., Sughosh, R., KM Jyoti, S., 2014. Biosynthesis of nanoparticles from ficusbenjamina (fig tree) and comparing AgNP's synthesized by cocktails of plant extracts. Int. Res. J. Biol. Sci. 3 (5), 2278–3202.
Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P., Misra, A., 2009. Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloid. Surface. Physicochem. Eng. Aspect. 339, 134–139.
Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M., 2006. Synthesis of gold nanotriangles and silver nanotriangles using Aloe vera plant extract. Biotechnol. Prog. 22, 577–579.
Chaudhry, Q., Castle, L., 2011. Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries. Trends Food Sci. Technol. 22, 595–603.
Clarke, Garry, Ting, Kang Nee, Wiart, Christophe, Fry, Jeffrey, 2013. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants (Basel, Switzerland) 2 (1), 1–10.
Durán, N., Marcato, P.D., De Souza, G.I.H., Alves, O.L., Esposito, E., 2007. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J. Biomed. Nanotechnol. 3, 203–208.
Fatimah, I., 2016. Green synthesis of silver nanoparticles using extract of Parkia speciosa Hassk pods assisted by microwave irradiation. J. Adv. Res. 7, 961–969.
Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O., 2000. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed. Mater. Res. 52, 662–668.
Ghaedi, M., Yousefinejad, M., Safarpoor, M., Zare Khafri, H., Purkait, M.K., 2015. Rosmarinus officinalis leaf extract mediated green synthesis of silver nanoparticles and investigation of its antimicrobial properties. J. Ind. Eng. Chem. 31, 167–172.
Huang, J., Chen, C., He, N., Hong, J., Lu, Y., Qingbiao, L., Shao, W., Sun, D., Wang, X.H., Wang, Y., Yiang, X., 2007. Biosynthesis of silver and gold nanoparticles by novel sun dried Cinnamomum camphora leaf. Nanotechnology 18, 105–106.
Ibrahim, H.M.M., 2015. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. J. Radiat. Res. Appl. Sci. 8, 265–275.
Jha, Anal K., Prasad, K., Prasad, Kamlesh, Kulkarni, A.R., 2009. Plant system: nature's nanofactory. Colloids Surfaces B Biointerfaces 73 (2), 219–223.
Kalaiyarasan, T., Bharti, V.K., Chaurasia, O.P., 2017. One pot green preparation of Seabuckthorn silver nanoparticles (SBT@AgNPs) featuring high stability and longevity, antibacterial, antioxidant potential: a nano disinfectant future perspective. RSC Adv. 7, 51130–51141.
Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T., Mohan, N., 2010. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surfaces B Biointerfaces 76, 50–56.
Kokura, S., Handa, O., Takagi, T., Ishikawa, T., Naito, Y., Yoshikawa, T., 2010. Silver nanoparticles as a safe preservative for use in cosmetics. Nanomedicine 6, 570–574.
Kumar, C.M.K., Yugandhar, P., Savithramma, N., 2016. Biological synthesis of silver nanoparticles from Adansonia digitata L. fruit pulp extract, characterization, and its antimicrobial properties. J. Intercult. Ethnopharmacol. 5, 79–85.
Kumar, P., Singh, P., Kumari, K., Mozumdar, S., Chandra, R., 2011. A green approach for the synthesis of gold nanotriangles using aqueous leaf extract of Callistemon viminalis. Mater. Lett. 65, 595–597.
Li, S., Shen, Y., Xie, A., Yu, X., Qiu, L., Zhang, L., Zhang, Q., 2007. Green synthesis of silver nanoparticles using Capsicum annuum L. extract. Green Chem. 9, 825–858.
Mulvaney, P., 1996. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12, 788–800.
Nayak, D., Pradhan, S., Ashe, S., Rauta, P.R., Nayak, B., 2015. Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. J. Colloid Interface Sci. 457, 329–338.
Nadkarni, K.M., Nadkarni, A.K., 1955. Indian Materia Medica.
Navaladian, S., Viswanathan, B., Viswanath, R.P., Varadarajan, T.K., 2006. Thermal decomposition as route for silver nanoparticles. Nanoscale Res. Lett. 2, 44–48.
Otari, S.V., Patil, R.M., Nadaf, N.H., Ghosh, S.J., Pawar, S.H., 2012. Green biosynthesis of silver nanoparticles from an actinobacteria Rhodococcus sp. Mater. Lett. 72, 92–94.
Peraza-Sánchez, S.R., Chai, H.-B., Shin, Y.G., Santisuk, T., Reutrakul, V., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Kinghorn, A.D., 2002. Constituents of the leaves and twigs of Ficus hispida. Planta Med. 68, 186–188.
Rastogi, R.P., Mehrotra, B.N., Sinha, S., Seth, R., Central Drug Research Institute (India), Council of Scientific & Industrial Research (India). Publications & Information Directorate, 2001. Compendium of Indian Medicinal Plants.
Saha, J., Begum, A., Mukherjee, A., Kumar, S., 2017. A novel green synthesis of silver nanoparticles and their catalytic action in reduction of Methylene Blue dye. Sustain. Energy Res. SER 27, 245–250.
Saif, S., Tahir, A., Chen, Y., 2016. Green synthesis of iron nanoparticles and their environmental applications and implications. Nanomaterials (Basel)(6).
Shankar, S.S., Ahmad, A., Rai, A., Sastry, M., 2004. Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles by using neem (Azadirachta indica) leaf broth. J. Colloid Interface Sci. 275, 496–502.
Sharma, V.K., Yngard, R.A., Lin, Y., 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci. 145, 83–96.
Smitha, S.L., Nissamudeen, K.M., Philip, D., Gopchandran, K.G., 2008. Studies on surface plasmon resonance and photoluminescence of silver nanoparticles. Spectrochim. Acta Mol. Biomol. Spectrosc. 71, 186–190.
Sreeram, K.J., Nidhin, M., Nair, B.U., 2008. Microwave assisted template synthesis of silver nanoparticles. Bull. Mater. Sci. 31, 937–942.
Starowicz, M., Stypuła, B., Banaś, J., 2006. Electrochemical synthesis of silver nanoparticles. Electrochem. Commun. 8, 227–230.
Sun, L., Yin, Y., Lv, P., Su, W., Zhang, L., 2018. Green controllable synthesis of Au–Ag alloy nanoparticles using Chinese wolfberry fruit extract and their tunable photocatalytic activity. RSC Adv. 8, 3964–3973.
Vilchis-Nestor, A.R., Sanchez-Mendieta, V., Camacho-Lopez, M.A., GomezEspinosa, R.M., Camacho-Lopez, M.A., Arenas-Alatorre, J.A., 2008. Solventless synthesis and optical properties of Au and Ag nanoparticles using Camellia sinensis extract. Mater. Lett. 62, 3103–3105.
Vigneshwaran, N., Kathe, A.A., Varadarajan, P.V., Nachane, R.P., Balasubramanya, R.H., 2007. Functional finishing of cotton fabrics using silver nanoparticles. J. Nanosci. Nanotechnol. 7, 1893–1897.
Yu, D.-G., 2007. Formation of colloidal silver nanoparticles stabilized by Na –poly(γ-glutamic acid)–silver nitrate complex via chemical reduction process. Colloids Surfaces B Biointerfaces 59, 171–178.