Int. J. Pharm. Investigation, 2020;10(3):312-319 Original Article

Green Synthesis of Silver Nanoparticles by using Stem, Leaves and Fruits Extracts of Umber (*Ficus racemosa*)

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ABSTRACT

Objectives: In this study, silver nanoparticles (AgNPs) were synthesized using healthy plant parts like fruits, stem and leaves of *Ficus racemosa*. **Methods:** The collected plant samples were processed by cleaning, drying and grinding. Bioactive compounds were extracted by using Soxhlet extraction method. The 10mg of plant extract was added to 1mM $AgNO_3$ solution and incubated at 80°C for 3min. The UV-Vis spectroscopy was used for characterization of produced AgNPs. The synthesis process was optimized for pH (3 to 9), plant extracts conc. (0.5, 1, 2, 4 and 8mg/2ml), incubation temp. (50°C, 60°C upto 100°C) and silver salt conc. (0.125, 0.25, 0.50, 1.0 and 2mM). Finally the antimicrobial and seed germination ability of synthesized AgNPs was determined. **Results:** The λ_{max} values were found to be 464.50nm, 422nm and 489nm for fruits, stem and leaves respectively. FTIR spectra of control and test were determined which showed the different functional groups in the synthesized AgNPs. The optimum pH (9, 8 and 8), plant extracts conc. (8, 4 and 8mg), incubation temp. (80°C, 90°C and 100°C) and silver salt conc. (2mM, 1.5mM and 2mM) for fruit, stem and leaves AgNPs were determined. The selective NPs showed notable antibacterial activity against Gram positive bacteria like *S. equorum* and *B. subtilis* and significant effect on wheat (*Triticum aestivum* L.) seed germination. **Conclusion:** The green synthesis process was found to be time saving and effective approach for the synthesis of the AgNPs from different plant parts. Produced NPs have potential applications in therapeutics and agriculture.

Key words: AgNPs, Antibacterial activity, FTIR, *B. subtilis, S. equorum.*

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DOI: 10.5330/ijpi.2020.3.56

INTRODUCTION

Nanotechnology is one of the key technologies of the 21st century.[1] The term nanotechnology is defined as the fabrications, manipulation and utilization of materials at a scale smaller than 1 mm.[2]

The nanoparticles are generally classified into the organic, inorganic, carbon based and composite based nanomaterials. Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide based nanoparticles are generally categorized as inorganic nanoparticles.[3] Almost all the metals can be synthesized into their nanoparticles.[4]

Silver has wide range of *in vitro* and *in vivo* applications and reported to be effective antimicrobial agent that exhibits low toxicity in humans.[5]

There are physical, chemical and biological methods used for nanoparticles synthesis but most widely recommended is green synthesis, which is an emerging area in the field of bio-nanotechnology which is a cheap and ecofriendly alternative to conventional methods. Green synthesis employ environmental friendly reagents as reducing and stabilizing agent.[6] Biosynthesis of nanoparticles by micro-organisms is a green and eco-friendly technology.[7]

Green synthesized nanoparticles reported to have diverse nature and more stability. General phenomenon of nanoparticles biosynthesis includes bioreduction of metals upon interaction with plant extracts. Plants metabolites like proteins, phenolic acids, polyphenols, alkaloids, terpenoids and sugars are generally used in metal ions reduction into nanoparticles.[8] The micro-organisms like bacteria and fungi are used for nanoparticles synthesis. The easy availability, non-toxic nature and diversity make plants as a best choice for nanosilver synthesis.[9]

In recent times, the plant mediated synthesis of nanoparticles is an emerging process due to its several advantages. Various parts of plants such as leaf, stem, root, flower, fruit and seed were utilized for the nanoparticles synthesis. Other materials related to the plants were also used for the synthesis of the nanoparticles like hull, peel and seed of the fruits. The biological compounds present in the plant parts were act as the reducing as well as capping or stabilizing agent in the process.[10]

Ficus racemosa plant is reported medicinal plant as it having various properties like antidiabetic,[11] larvicidal[12] antioxidant and cytotoxic.[13]

Tender fruits of this plant used in dry cough, disorders of kidney and spleen, in treatment of leucorrhoea, burning patient's treatment and digestive system disorders. They are also useful in treatment of miscarriage, spermatorrhoea, epididymitis and cancer.[14,15]

The leaf buds of plant are used for skin application. A leaves extract is also used for washing wounds. The bark infusion is also used as a mouth wash in gums problem and in dysentery, glandular swelling, in treatments of chronic wounds and haemoptysis.[16]

To date, there is no significant work was done on application of aqueous leaf and stem extracts of *Ficus racemosa* in nanoparticles synthesis but unripened fruit extract was used for AgNPs synthesis.[17]

Hence, in the present work a systematic approach was used to design protocol to synthesize and characterize silver nanoparticles from different

extract of the *Ficus racemosa* for evaluation of their antibacterial activity and effect on seed germination.

MATERIALS AND METHODS

Sample collection

The healthy plant part samples like fruits, stem and leaves were collected from Baramati, Pune, Maharashtra, in sterile polythene bags, brought to laboratory and stored until use.

Processing of the samples

The collected samples were cleaned thoroughly with fresh water to remove adhering debris and associated biota.[17] After cleaning; the samples were dried in shade at RT for two weeks. Drying is important for the pre-extraction preparation of plant materials.[18] After drying; the samples were grind for making powder.[19]

Soxhlet extraction

The Soxhlet extractor used in this study setup consists of a condenser with cooling water inlet and outlet, an extractor with siphon tube and distillation path, a round bottom flask, heat source and thimble for holding sample. In this study, ethanol was selected as a solvent. Total 150 ml of the ethanol along with boiling stone was added in the round bottom flak followed by assembling the apparatus. The 75°C temp. was selected for the extraction process (boiling point of the solvent). The thimble (of Whatmann filter paper no. one) containing 15g of powder sample of plant was placed in extractor. The heating process was carried out for 24 hr. to complete 16 cycles. The extract collected was subjected to evaporation and dried extract stored in low temp. until use.

Green synthesis of AgNPs

For preparation of 1mM silver nitrate solution 0.017g silver nitrate powder was added to 100 ml distilled water. Typical reaction mixture contained 10mg of stem or fruits or leaves extract in 2ml of silver nitrate solution. The mixture then subjected to incubation at 80°C for 3 min.[20] After incubation the visible colour change was the indication of NPs synthesis.

Characterization of AgNPs

All the three types of nanoparticles produced were subjected to characterization by using UV-Vis Spectrophotometer (Elico-SL 210 UV Vis. Spectrophotometer). The absorption spectrum of the NPs was measured in the range of 200 to 800nm by using $AgNo_3$ solution as a blank.[21] To determine the functional groups present in bioactive compounds and their possible involvement in the synthesis process, plant powder (fruits, stem and leaves) along with respective AgNPs were subjected to FTIR analysis. The spectra were collected at spatial resolution of $4cm^{-1}$ in the transmission mode ($4000- 400cm^{-1}$) using a Shimadzu FTIR spectrophotometer-FTIR 8400.[20]

Optimization of AgNPs by classical method

Different parameters were studied for optimization viz. pH, plant extract conc., incubation temp. and silver salt conc. The effect of pH on NPs formation was evaluated by using pH range from 3 to 9. The plant extract content was varied (0.5, 1, 2, 4 and 8mg) while keeping the silver nitrate conc. at 1mM.[20] The effect of temp. on synthesis was evaluated by incubating plant extract with 1 mM $AgNO_3$ at different temp. viz. 50°C, 60°C, 70°C, 80°C, 90°C and 100°C. To check the effect of silver nitrate conc. on synthesis process, plant extracts were mixed with different conc. of silver nitrate (0.125, 0.25, 0.50 up to 2mM).

Antimicrobial effect against MDR

The activities of produced NPs against potent pathogens were studied by using well diffusion method against bacterial strains *B. subtilis* and *S. equorum*. The pure cultures of selected strains were maintained on nutrient agar (NA) slants containing peptone - 1.0g, malt extract - 0.3g, sodium chloride - 0.5g, agar - 2.5g per 100ml of distilled water. The bacterial suspensions (100µl) containing 10^4 cells ml^{-1} were spread on NA plates. Freshly prepared and optimized 50µl fruit AgNPs (at 9 pH, 8mg plant extract, 80°C temp. and 2 mM $AgNO_3$ conc.) were added into the wells that were made in the seeded plates.[20] The silver nitrate solution was used as control.[22] The plates were initially incubated for 15 min. at 4°C for free diffusion and later transfer to incubator (37°C for 24 hr) for the incubation purpose. After incubation the diameter of zone of inhibition was recorded.[20]

Effect of AgNPs on seed germination

Healthy and equal weight seeds *T. aestivum* L. variety Lokvan were collected from agriculture store (Jagdamba seeds suppliers) from Baramati for determination of NPs effect on wheat germination.

Germination assay

For surface sterility, seeds were treated with 10% sodium hypochlorite solution for 10 min., then treated with nanoparticle suspensions for about 2 hr.[23] A filter paper piece was kept in Petri dish onto which 5 ml test medium was added. 5 seeds were then placed in each plate by keeping one cm distance between each seed.[24] Finally all the plates were sealed and placed in an incubator for 5 days. After incubation percent seed germination was calculated and root length and shoot length of seedling was also measured.[25]

RESULTS

Selection of plant

Different parts of *Ficus racemosa* plant were selected for the present study as very few reports are available on the application of this plant in nanobiotechnology. The plant is easily available in various regions of Baramati, Pune, Maharashtra.

Collection of plant

The healthy plant parts like fruits stem and leaves were collected from various region of Baramati, Pune.

Processing of the collected samples

Cleaning

Collected samples thoroughly washed with fresh water to remove adhering debris and associated biota and brush was used for the removal of the epiphytes with distilled water.

Drying

The drying process is important for the pre-extraction preparation of samples. The samples was shade dried, because overheat can lose the volatile bioactive compounds from plant materials and many light sensitive constituents may lose in light condition.

Grinding

Grinding is the essential step to obtain a homogenous and uniform sized sample powders, as smaller particle size has greater surface area of the powdered particles. In the present study, grinder was used for this purpose.

Soxhlet extraction

For the analysis and characterization, chemical components from the plant materials can be effectively extract out by using Soxhlet extraction. We have successfully used the same method for extraction of fruit, stem and leaves samples.

There are various extraction methods used by the researchers for plant samples, which are classified as hot and cold extraction. The temp. sensitive compound from the sample can be extracted by cold extraction approach. It is the extremely effective, accurate and time saving approach for extraction. After extraction process the solvent was removed from round bottom flask and evaporated successfully to obtain a pure dried extract.

Green synthesis of AgNPs

After addition of three different extracts viz. stem, fruits and leaves separately into silver nitrate solution, the colour of reaction mixture turned into dark brown after three min of incubation, which indicate the synthesis of AgNPs. This change in colour indicates Ag^{+} reduction to Ag° due to the formation of crystals.

Characterization of AgNPs

UV-Visible spectroscopy

In the UV-Vis spectrum; a single, strong and broad surface plasmon resonance (SPR) peaks of AgNPs was observed in the range of 200 to 800nm. The obtained spectrum showed max. absorbance at 464.50nm, 422 nm and 489 nm of fruit, stem and leaf extract AgNPs respectively.

FTIR analysis

The functional groups of fruit, stem and leaf extract of *Ficus racemosa* plant were determined by using FTIR spectroscopy. The control spectra of each extract showed peaks which indicate the presence of number of biomolecules as shown in the Figure 1.

Various peaks observed after analysing test samples as AgNPs from fruit showed 14 absorption bands at 408, 435, 451, 472, 495, 1238, 1369, 1631, 1641, 3282, 3294, 3315, 3329 and 3340cm^{-1} as shown in Figure 1 (a and b). NPs from stem extract showed 15 different peaks at 414, 430, 439, 462, 489, 511, 1238, 1369, 1631, 1641, 3261, 3277, 3305, 3317 and 3325cm^{-1} as shown in Figure 1 (c and d). Whereas leaf NPs showed 6 different peaks at 451, 535, 572, 1632, 2080, 3279 cm^{-1} as shown in Figure 1 (e and f).

The control spectra (without silver nitrate) showing peaks reflecting a complex nature of the fruits, stem and leaf extract. The band pattern of the spectrum for the control and test samples was analyzed and compared as shown in figure 1. The peak present at around 2337 cm^{-1} was attributed to the N-H stretching or the C-O stretching vibrations. The peak located at 1641 cm^{-1} could be assigned to the C-O stretching in carboxyl or C-N bending in the amide group. Significant change in the band pattern indicate their probable role in the nanoparticle synthesis.

Optimization of AgNPs by classical method

The nanoparticles synthesis process was successfully optimized by classical one parameter at a time approach.

Effect of pH

The AgNPs produced from fruit and stem sample showed less colour change at acidic pH (3, 4, 5 and 6) but when pH shifted to basic (7, 8 and 9) the intensity of colour significantly increased. In case of leaf NPs, after incubation the colour intensity was high at 6, 7, 8 and 9. The spectroscopic analysis of reaction mixture of fruit NPs at pH 9 (λ_{max}-438nm), stem NPs at pH 8 (λ_{max}-345nm) and leaf NPs at pH 8 (λ_{max}-434.50nm) showed presence of AgNPs as showed in Figure 2.

Effect of extract conc.

In different conc. of plant extract (0.5, 1, 2, 4 and 8mg), the colour produced varies in synthesis process. The drastic colour variation was observed when extract conc. was varied in fruit and stem reaction mixture. As the quantity of fruit and stem extract increased, the colour intensity decreased. The colour intensity found to be independent on leaf extract concentrations. The spectroscopic analysis of fruit (8mg), stem (4mg) and leaves (8mg) NPs showed max absorbance at 464.50nm, 422nm and 489nm respectively as showed in Figure 3.

Effect of reaction temperature

The reaction mixture incubated at different temp. (50 to 100°C), increase in sharpness of absorbance peak was observed. Fruit, stem and leaves NPs showed reddish brown, yellowish brown and dark brown colour respectively at different incubation temp. The max SPR peak intensity of fruit, stem and leaves NPs was detected at 80°C (λ_{max}-398nm), 100°C (λ_{max}-368.50nm) and 90°C (λ_{max}- 419.50 nm) respectively as shown in the Figure 4.

Effect of silver nitrate conc.

The effect of the silver salt on synthesis process was determined by varying the conc. of silver nitrate (0.125, 0.25, 0.5, 1.0 to 2.0 mM). Fruit and stem extract NPs showed yellowish brown to reddish brown colour after incubation and max. absorbance found at 2.0 mM conc. ($\boldsymbol{\lambda}_{max}$- 433nm) and 1.5mM conc. (λ_{max}- 419nm) respectively. NPs from leaf extract showed colourless to dark brown colour at 1.25 to 2.0 mM $AgNO_3$ conc. and max absorbance obtained at 2.0 mM conc. ($\boldsymbol{\lambda}_{max}$-489nm) as shown in the Figure 5.

Antibacterial activity of AgNPs

The effect of the synthesized AgNPs (fruit) on potent bacterial pathogens *B. subtilis* and *S. equorum* was determined by using well diffusion technique.

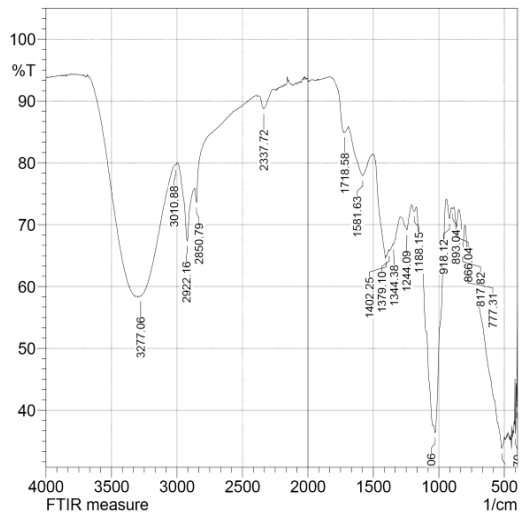
NPs synthesized at optimum salt conc. showed highest and temp optimized NPs showed lowest activity against *S. equorum* but both NPs showed similar activity against *B. Subtilis*. NPs produced at optimum pH showed highest activity against *B. subtilis* and NPs synthesized at optimum extract conc. showed similar effect against both MDR pathogens (table 1).

Effect of AgNPs on seed germination

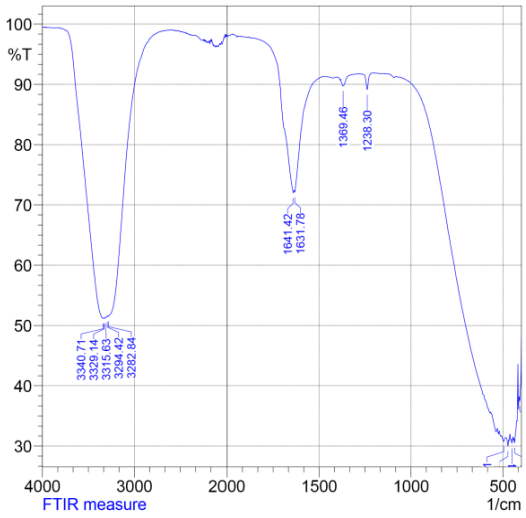
The AgNPs shows significant effect on morphological growth which was measured in terms of root and shoot length of *T. aestivum* L. The

Table 1: Antimicrobial activity of AgNPs against MDR. Comparative account of the effect of different physical parameter on AgNPs.

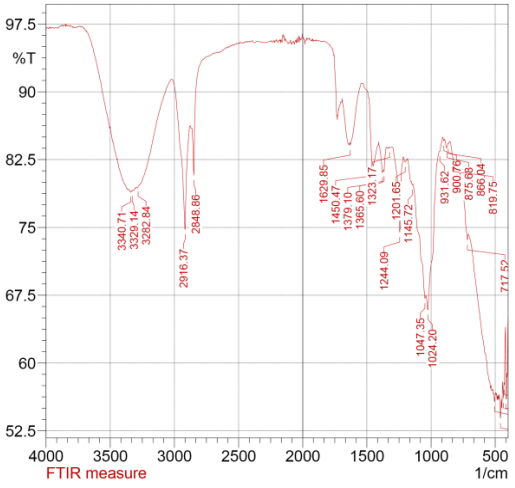
Sr. No	Parameter	Zone of inhibition (in mm) Control ($AgNO_3$ solution)	Zone of inhibition (in mm) fruit NP solution	Zone of inhibition (in mm) Control ($AgNO_3$ solution)	Zone of inhibition (in mm) fruit NP solution
		S. equorum		*B. subtilis*	
1	Temp	8	10	10	12
2	Extract	8	11	10	11
3	pH	9	12	9	15
4	Salt	9	13	10	12



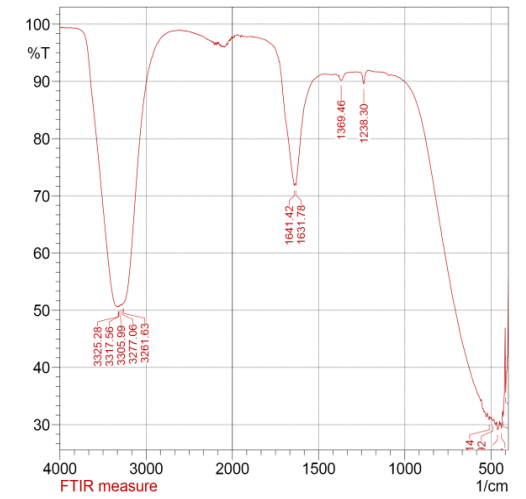
a (control)



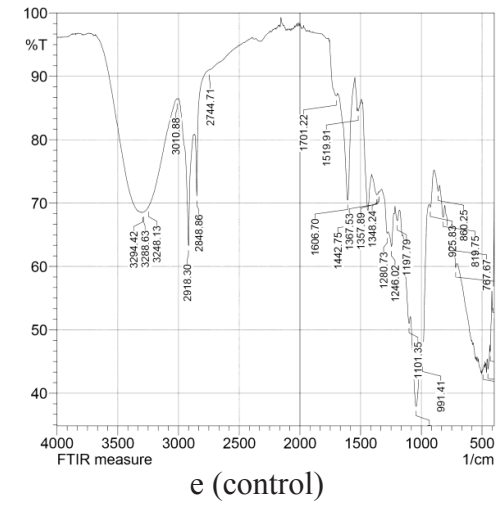
b (test)



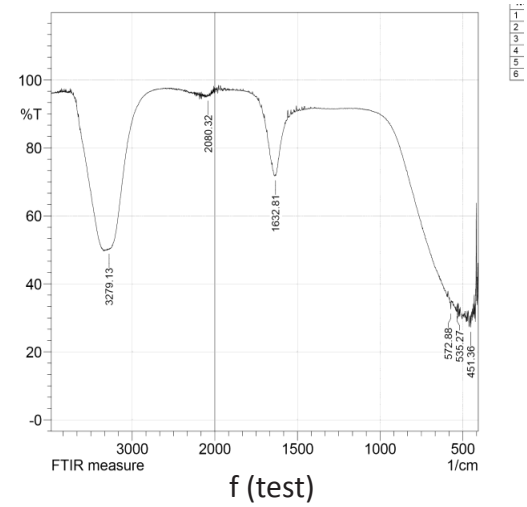
c (control)



d (test)



e (control)



f (test)

Figure 1: FTIR analysis of fruit (a and b),stem (c and d), leaf (e and f) extract.

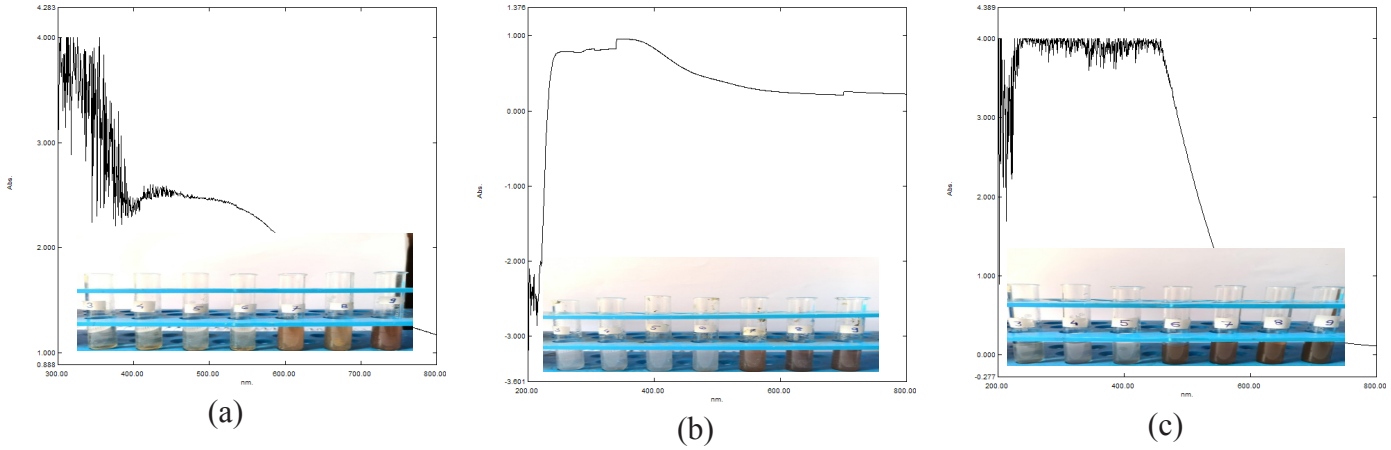


Figure 2: The spectroscopic analysis of NPs synthesized from extract of (a) fruit at pH 9 (b) stem at pH 8 and (c) leaf at pH 8. Tubes showing the effect of different pH viz. 3, 4, 5, 6, 7, 8 and 9 on colour intensities of produced NPs by incubating fruit, stem and leaves extract (10mg) with 1 mM $AgNO_3$ solution. The mixture was incubated at 80°C for 3 min.

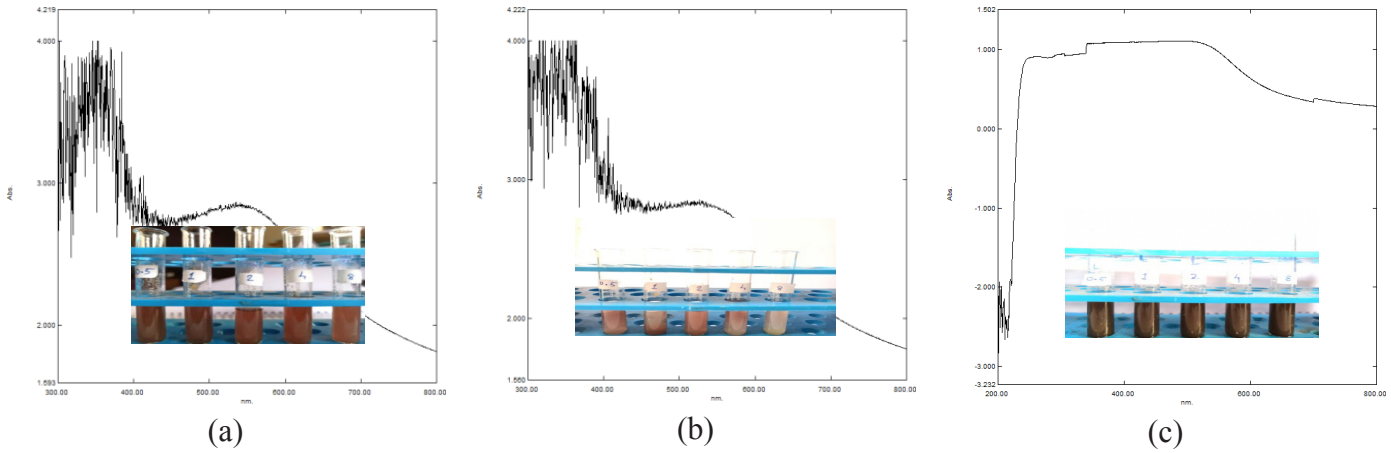


Figure 3: The spectroscopic analysis of NPs synthesized from (a) fruit at 8 mg (b) stem at 4 mg and (c) leaf at 8 mg extract conc. Tubes showing the effect of different conc. of plant extract viz. 0.5, 1, 2, 4 and 8mg on colour intensities of NPs produced, when different extract conc. was mixed with 1 mM AgNO3 solution and incubated at optimum pH at 80°C temp. for 3 min.

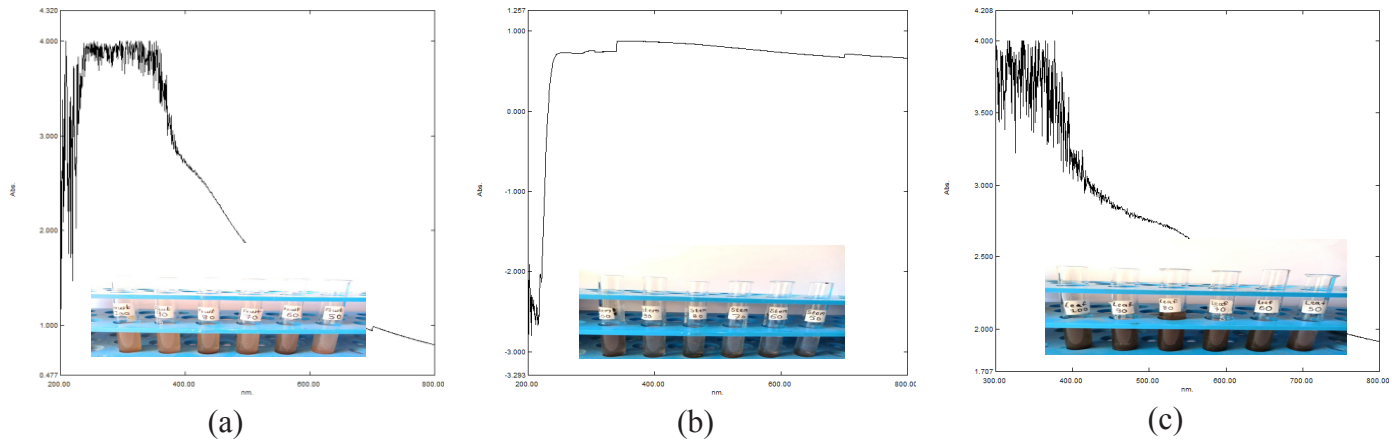


Figure 4: The spectroscopic analysis of NPs synthesized from extract of (a) fruit at 80°C (b) stem at 100°C and (c) leaf at 90°C temperature. Tubes showing the effect of different temp. viz. 50°C, 60°C, 70°C, 80°C, 90°C and 100°C on colour intensities of NPs produced by incubating fruit (8mg), stem (4mg) and leaves (8mg) with 1 mM $AgNO_3$ for 3 min.

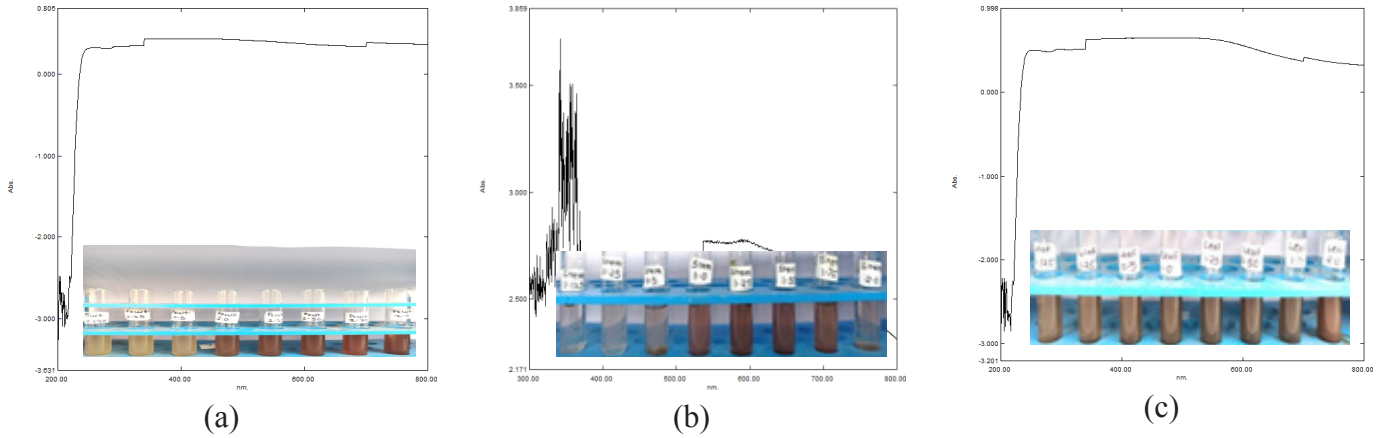
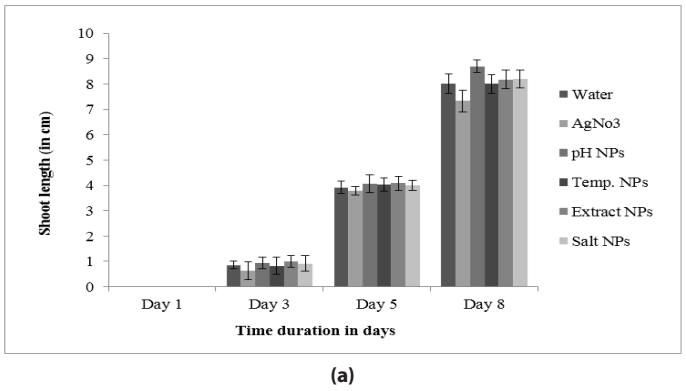
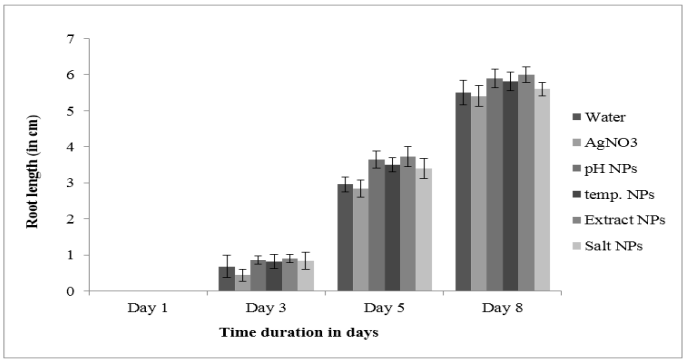


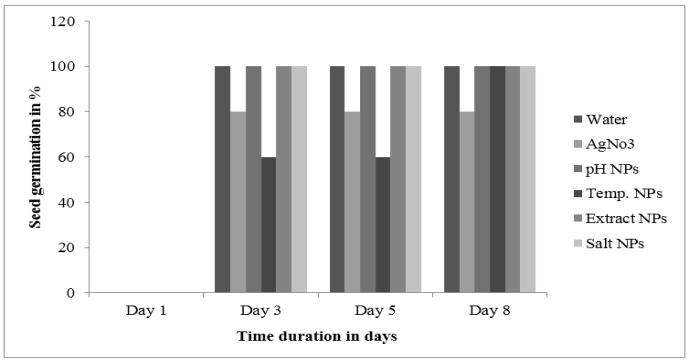
Figure 5: The spectroscopic analysis of NPs synthesized from extract of (a) fruit at 2 mM (b) stem at 1.5 mM and (c) leaf at 2mM silver nitrate conc. Tubes showing the effect of different conc. of silver nitrate 0.125, 0.25, 0.5, 1.0 to 2.0 mM. on colour intensities of NPs produced when incubated with fruit (8mg), stem (4mg) and leaves (8mg) extract for 3 min at 80°C, 100°C and 90°C respectively.



(a)



(b)



(c)

Figure 6: Effect of NPs on (a) shoot length, (b) root length and (c) germination percent of wheat plant

seeds which are treated with selective (fruit) AgNPs solution showed considerable increase in root and shoot length as compared to control. The notable growth of plantlets was observed on the third day.

In the present study, NPs produced at optimized pH, plant extract, temp. and salt conc. showed highest shoot length 8.7±0.254, 8.18±0.356,8.0±0.360 and 8.2 ± 0.353 cm respectively on eighth day. NPs produced at optimized pH, plant extract, temp. and salt conc. showed highest root length 5.9 ± 0.254, 6.0 ± 0.223,5.82 ± 0.258 and 5.6 ± 0.187 cm respectively on eighth day.

Seed germination percentage of water and NPs produced at optimized pH, extract, temp. and salt was 100% but for $AgNO_3$ solution it was 80%.

DISCUSSION

The *Ficus racemosa* plant has already reported to have various medicinal properties like antidiarrheal activity[26] as well as antifungal activity.[17] and CNS depressant activity.[27] The leaf of *F. racemosa* used for synthesis of zinc oxide NPs (ZnO NPs) and it showed the good antibacterial activity on both Gram positive as well as Gram negative bacteria.[28] The stem sample was cleaned with distilled water before grinding to remove medium components.[29] The shade drying is mostly suitable than other drying methods.[30] Large surface area improves the contact of the powdered particles with the solvent used for extraction[19] and hence efficient extraction takes place. Grinding in mortal and pestle do not have sufficient results as compared to mixer grinding.[31]

The Soxhlet extraction method can be effectively used to extract compound with more efficiency.[32] Soxhlet extraction is most common extraction technique in which high temp. facilitate the extraction of target compound; moreover, the repeated contact of solvent with extract can enhance the extraction yield.[33] Root extracts of *Diospyros paniculata* were soxhlet extracted with methanol for synthesizing AgNPs.[34] Crude plant extract was prepared by soxhlet extraction method by using methanol as menstrum of *Eucalyptus hybrid* (safeda) leaf.[35]

The colour change was caused by surface plasmon resonance (SPR) of AgNPs in the visible region.[36] The green unripened papaya (*Carica papaya* L.) fruit extract,[37] *Salvadora persica* stem extract[38] and aqueous leaf extract of *Carica papaya*[39] used for the formation of AgNPs. The colour change indicated the formation of AgNPs.

AgNPs can be synthesized using the *Crataegus douglasii* fruit extract and λ_{max} was found 450nm.[40] The AgNPs synthesized from *Boswellia ovalifoliolata* stem bark has showed absorbance peak at 430 nm.[41] The

leaf extract of *Eucalyptus oleosa* used for AgNPs synthesis and showed a sharp peak at 460nm (visible region) which was an evidence for the formation of AgNPs.[42]

The peak in the range of 3200-3500cm^{-1} was assigned as -OH stretching in alcohol and phenolic compound with strong hydrogen bond.[43] The band at 1631cm^{-1} in the spectra corresponds to C-N and C-C stretching indicating the presence of proteins.[44] The presence of vibration at 1641 cm^{-1} representing that AgNPs is attached to proteins by carboxylate group.[45] All these vibrations and bends shows that this groups are bounded along with AgNPs.

The previous report of NPs produced from *Solanum nigrum* leaves optimization for different pH range (5, 7 and 9) found that increase in pH leads to shifting absorption maximum from low to high. So according to them the pH enhances the rate of reduction reaction.[44] Absorbance of peak was depend on size of the synthesized NPs. as with higher temp. Particle size may be smaller, which results into sharpness of the plasmon resonance band of AgNPs.[46] The observation is our findings were in agreement with the above finding. The colour intensity in reaction mixture is increased as per increasing the temp. It is well known fact that when the temperature is increased, the reactants are consumed rapidly eventually leading to synthesis of nanoparticles.[47] The AgNPs were prepared by using different conc. of silver nitrate solution with watermelon extract as reducing and capping agent and characterized by using UV-Visible spectroscopy which showed maximum yield of AgNPs at 2 mM.[48]

The AgNPs shows antibacterial activity because of (i) super oxide anions (O^{2-}) and hydroxyl radicals (OH), (ii) the Ag^{+} ions of AgNPs makes bonding with sulfhydryl groups which responsible for denaturation of proteins in the bacteria[49] and (iii) release of Ag^{+} ions from the AgNPs which simply penetrate into the cell wall that kill bacteria. The previous report for antimicrobial activity of *Chenopodium murale* leaf extract, silver nitrate ($AgNO_3$) and AgNPs was determined. The results showed that leaf extract did not exhibit notable antimicrobial activity against *S. aureus*. Silver nitrate showed notable effect and AgNPs showed greatest activity against tested micro-organisms.[50]

The previous study report on TiO_2 NPs showed uptake and impact on plants (wheat and rapeseed) in hydroponics conditions either through root or leaf exposure.[51] In the previous report the effect on germination after exposure of wheat (*T. aestivum*) seeds to silver, copper and iron NPs was studied under laboratory conditions. Germination percentage, root and shoot length were calculated. The previous reports suggest decrease percent germination due to exposure of silver and copper NPs while highest percent germination and root and shoot development on application of iron NPs. So, according to them copper has inhibitory while iron has stimulatory effect on wheat germination and growth.[52]

The germination of *Cicer arietimum* under the influence of ZnO and TiO_2 NPs was determined. So, this study revealed that germination was enhanced by TiO_2 than ZnO NPs. Morphological characters showed that ZnO NPs have negative effect on root and shoot length.[53]

CONCLUSION

Fruit, stem and leaf of *Ficus racemosa* plant were successfully utilized for the consistent and quick synthesis of AgNPs. So, from this we conclude that, these three healthy plant parts have a great potential for synthesizing NPs with a vast range of applications. Variation in reaction condition affected nanoparticles synthesis. The colour intensity was pH dependent. These reaction mixtures showed variation in colour intensity and characterization of AgNPs by using UV-Visible spectra. FTIR spectra confirmed the NPs were covered by plant compounds. The synthesized AgNPs have displayed antibacterial activity towards the pathogenic bacteria as well as the significant effect on seed germination. The produced NPs are eco-friendly and having rapid way for the synthesis providing a cheap and an efficient method of AgNPs synthesis. These NPs possess the biomedical applications and will play notable role in medical field in near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Min: minute; **conc:** concentration; **mg:** milligram; **temp:** temperature; **AgNPs:** Silver nanoparticles; **nm:** nanometer; **FTIR:** Fourier Transform Infrared; **mm:** millimeter; **mM:** milliMolar; **NPs:** nanoparticles; **TiO_2:** Titanium dioxide; **Max:** maximum; **CNS:** Central nervous system.

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Article History: Submission Date : 18-06-2020; Revised Date : 18-08-2020; Acceptance Date : 10-09-2020.
Cite this article: Kadam SD, Shinde SP, Chavan TS, Phatake YB, Mane SG, Dhawan SS, *et al.* Green Synthesis of Silver Nanoparticles by using Stem, Leaves and Fruits Extracts of Umber (*Ficus racemosa*). Int. J. Pharm. Investigation. 2020;10(3):312-9.