Optimization Of Process Parameters for Synthesis of Silver Nanoparticles Using Leaf Extract of Tridax Procumbent and Its Biotechnological Applications

Laxmikant R. Patil*, Anil R. Shet, Arati G. Lohar, Gururaj B.Tennalli, Sharanappa A., V. S. Hombalimath, Umesh Deshannavar

Abstract: Green synthesis of nanoparticles have acquired a lot of importance because of their cost effectiveness and environmental friendly nature. In this work, optimization of process parameters for the synthesis of silver nanoparticles (AgNPs) using leaf extracts of Tridax procumbens was done. Ultraviolet-Visible (UV-Vis) spectrophotometer was used for the confirmation of AgNP synthesis. The effect of process parameters like quantity of leaf extract, silver nitrate ($AgNO_3$) concentration, incubation time, temperature and pH were studied. The optimum conditions were found to be 1ml of leaf extract, 10mM concentration of silver nitrate, pH of 9, incubation time of 180minutes and temperature of 40^oC. The synthesized AgNPs were examined for antimicrobial property against two Gram-positive bacteria and two Gram-negative bacteria. Highest antibacterial activity was noticed for Gram negative bacterium Pseudomonas aeruginosa. Antioxidant properties were analyzed by DPPH and H_2O_2 assay and were found to be significant for the synthesized AgNPs. Further AgNPs were used for the qualitative detection of Hydrogen peroxide.

Keywords: Tridax procumbens, Phytochemical screening, Silver nanoparticles, UV-spectroscopy, Antimicrobial activity, Antioxidant activity, H_2O_2 detection.

1. INTRODUCTION

Nanotechnology is an important area of investigation which deals with production of nanoparticles of the size ranging between 1 to 100nm [1]. Among different metallic nanoparticles, AgNPs have gained a lot of importance because of their distinct properties like antimicrobial, anti-inflammatory, anticancer, antiviral activity and thermal conductivity [2, 3]. There are many advantageous of green synthesis of nanoparticles over physical and chemical synthesis such as eco-friendly nature, cost effectiveness, low operating conditions and use of non-toxic chemicals [4, 5]. The AgNP synthesis using plant source is advantageous over microbial synthesis due to reduction in cost of microbial isolation, maintenance of asceptic conditions, culture media preparation and carrying out fermentation [6]. Plant extract provide natural capping and reducing agents for AgNP synthesis [7, 8]. Hence owing to the advantages of plant mediated nanoparticle synthesis, the present work was focused at AgNP synthesis from leaf extract of Tridax procumbens and its biotechnological applications. Tridax procumbens contains variety of phytochemicals, like polyphenols, alkaloids, flavonoids, which act as the source of capping and/or reducing agent in the AgNP synthesis [9].

2 MATERIALS AND METHODS

2.1 Materials

The leaves of the plant Tridax procumbens were collected from the local garden. Silver nitrate was procured from SRL Pvt Ltd. Nutrient broth and Agar powder was obtained from HiMedia Laboratories. UV visible spectrum was recorded using spectrophotometer (UV shimadzu 1700).

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2.2 Preparation of plant extract

5grams of Tridax procumbens plant leaves were taken and cleaned with distilled water. Then it was chopped and added to distilled water (100ml) and boiled using hot plate. The leaf extract was filtered (whatman's paper), stored in refrigerator.

2.3 Phytochemical screening

Phytochemical screening of leaf extract of Tridax procumbens was conducted for the qualitative detection of various phytochemicals like sterols, polyterpenes, polyphenols, flavonoids, quinine substances, saponosides, tannins, alkaloids, glycosides, carbohydrates and triterpenes. All phytochemical tests were carried according to standard assay protocols [10].

2.4 Preparation of $AgNO_3$ solution

5mM $AgNO_3$ solution was prepared by addition of 0.085 grams of $AgNO_3$ in 100ml of distilled water. It was stored in amber color reagent bottle away from direct sunlight.

2.5 Biosynthesis of AgNPs

Green synthesis of AgNPs was performed by addition of different concentration of $AgNO_3$ to different volumes of leaf extract. Prepared solution was kept in dark chamber for incubation of 1hr. The color change was observed from colorless to brown. One factor at a time (OFAT) studies were conducted to observe the influence of concentration of $AgNO_3$ (1mM, 5mM & 10mM), amount of leaf extract (0.1ml, 0.5ml & 1.0ml), pH (5, 7, 9), incubation time (120, 180, 240 minutes), temperature (10, 20, 40, 60, 80, 100^o C) on AgNP synthesis.

2.6 Characterization of AgNPs

2.6.1 UV-Visible spectroscopy

The synthesis of AgNPs was visually evidenced by the change in color from colorless to brown. UV-Visible spectroscopy measurements confirmed the synthesis of AgNPs. Spectral data was recorded in the range of 200-800nm wavelength.

2.7 Application of silver nanoparticles

2.7.1 Antimicrobial activity

Culture slants of four bacteria, two gram positive: Staphylococcus aureaus & Micrococcus Luteus and two gram negative: Ecoli & Psedomonas aerogenosa were obtained from Department of Biotechnology, KLE Technological University, Hubballi. These slants were maintained at 4^{o}C with regular sub-culturing on Nutrient Agar (NA) slants every fortnight and used as master slants. Antimicrobial activity was carried out by streak plate disk diffusion method. Bacterial strains were streaked on nutrient agar plates using sterilized nichrome wire loop. Five disks were placed on each plate which represented the leaf extract (E), silver nitrate ($AgNO_3$) of concentration 5mM, silver nanoparticles (AgNPs), distilled water as negative control (NC) and chloramphenicol antibiotic (positive control-centre of the disk). After incubation for 24hrs at 37ºC, the plates were analyzed for zone of inhibition.

2.7.2 Antioxidant activity

The antioxidant activity was accomplished by DPPH and Hydrogen peroxide assay.

DPPH assay

The DPPH activity of synthesized AgNPs and plant extract were performed by UV-Vis spectrophotometer. The antioxidant property of AgNPs was measured using the DPPH free radical assay. In this assay, 2 ml of DPPH solution (dissolved in methanol at concentration of 0.1 mM) was mixed with 1mg/ml of test material and kept for incubation for 30 minutes in the dark. The absorbance was measured at a wavelength of 517 nm. Methanol was taken as blank. 1ml of methanol and 2 ml of DPPH were taken as control. 1mg/ml of Ascorbic acid was taken as standard. % Antioxidant activity was determined using the formula : (1 – sample absorbance / control absorbance) ×100.

Hydrogen peroxide assay

H_2O_2 antioxidant property was analyzed by UV-Vis spectrophotometer at 230nm. Antioxidant activity of AgNPs was expressed in terms of ascorbic acid (standard control) antioxidant equivalents. The % antioxidant property was determined using the following expression: Antioxidant activity (%) = [(x –y)/x] × 100, where 'x' = absorbance of the blank and 'y' = absorbance of the sample.

2.7.3 Detection of H_2O_2

To study the hydrogen peroxide detection by biogenic silver nanoparticles, 1mM, 10mM and 100mM concentration of H_2O_2 was prepared. 1 ml of H_2O_2 solution was mixed with the known concentration of AgNPs. The color of the nanoparticles changed from brown to colorless. Then UV-visible spectrum analysis was done for all the samples.

3 RESULTS AND DISCUSSION

3.1 Preparation of leaf extract

Fig. *1. Tridax procumbens plant and its leaf extract*

3.2 Phytochemical analysis of Tridax leaf extract

The presence of alkaloids, carotenoids, flavonoids, saponins, and tannins in Tridax procumbens leaf extract has been investigated. The phytochemical analysis of aqueous leaf extract are indicated in Table 1. Phytochemicals such as sterols, polyterpenes, polyphenols, flavonoids, quinine substances, saponosides, tannins, alkaloids, glycosides, carbohydrates and triterpenes were observed in the leaf extract. The presence of these bioactive components may play a major role in the reducing, capping and stabilization of AgNPs.

Table1. *Phytochemical analysis of leaf extract*

PHYTOCHEMICAL COMPOUND	CHLOROFORM	METHANOL	AQUEOUS EXTRACT
STEROLS AND POLYTERPENES	+	+	+
POLYPHENOLS	+	+	+
FLAVONOIDS	+	+	+
QUINONE SUBSTANCES	-	-	+
SAPONOSIDES	-	-	+
CATECHIN TANNINS	+	+	+
GALLIC TANNINS	-	+	+
ALKALOIDS	+	+	+
GLYCOSIDES	-	-	+
CARBOHYDRATES	+	+	+
TRITERPENES	-	-	-

3.3 Biosynthesis of AgNPs

Biosynthesis of AgNPs was done by Tridax leaf extract. $AgNO_3$ was added to the Tridax leaf extract which resulted into a color change from light yellow to dark brown within 120 minutes of the reaction. The color change is due to the presence of reducing agents (flavonoids and terpenoids) in the leaf extract [11]. UV-Vis spectral analysis was carried out to confirm the AgNP synthesis.

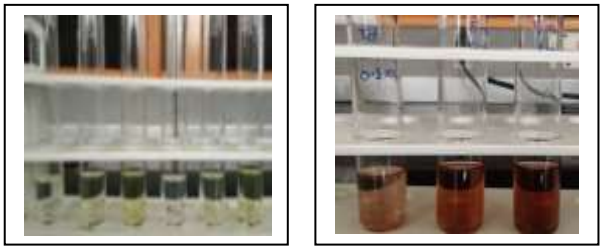

Fig.2*. Color change from colorless to dark brown indicating the formation of silver nanoparticles*

3.4 OFAT studies on biosynthesis of silver nanoparticles

3.4.1 Effect of amount of leaf extract on synthesis of AgNPs

The different leaf extract concentration (0.1ml, 0.5ml and 1.0ml) was added to aqueous $AgNO_3$ solution (1mM concentration). The color change took place from light yellow to darkish brown indicating synthesis of AgNPs. As the leaf extract concentration was increased, there was an increase in the absorbance. The increase in the absorbance indicates the synthesis of small sized AgNPs. All the results are in line with the reported literature [11, 12, 13].

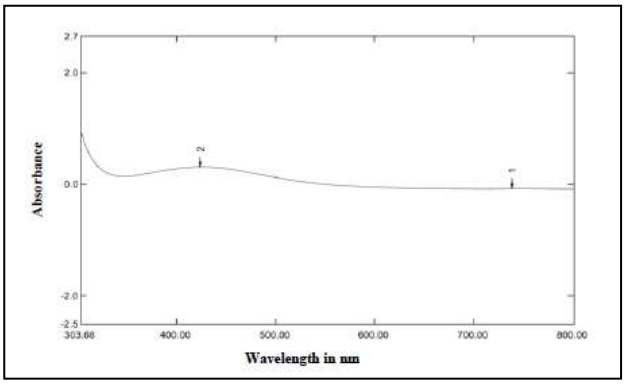

***Fig.3**. UV-Vis spectrum of the AgNP synthesized by 0.1ml of leaf extract*

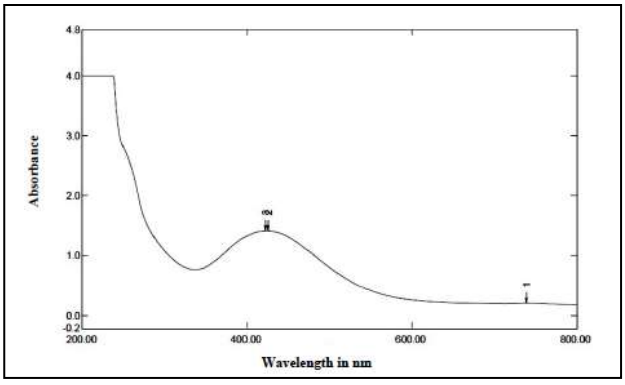

***Fig.4**. UV- Vis spectrum of the AgNP synthesized by 0.5ml of leaf extract*

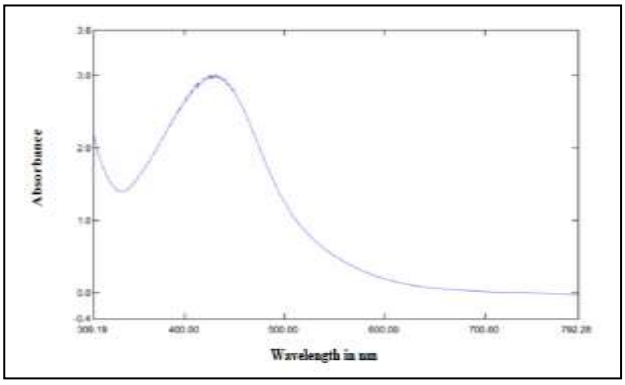

***Fig.5**. UV-Vis spectrum of the AgNP synthesized by 1.0ml of leaf extract*

3.4.2 Effect of $AgNO_3$ concentration on synthesis of AgNPs

Various $AgNO_3$ concentrations (1mM, 5mM and 10mM) were added to 0.1ml aqueous leaf extract solution. The color change took place from light yellow to darkish brown indicating AgNP formation. As silver nitrate concentration was increased, there was an increase in the absorbance. The increase in the absorbance indicates the synthesis of small sized AgNPs. All the results are in line with the reported literature [11, 12, 13].

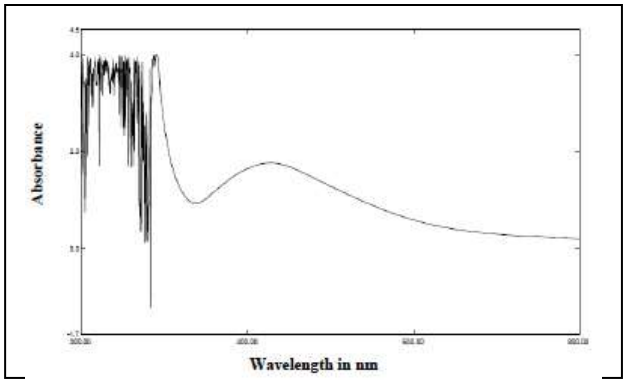

***Fig.6**. UV-Vis spectrum of the AgNP synthesized by 1mM $AgNO_3$ concentration*

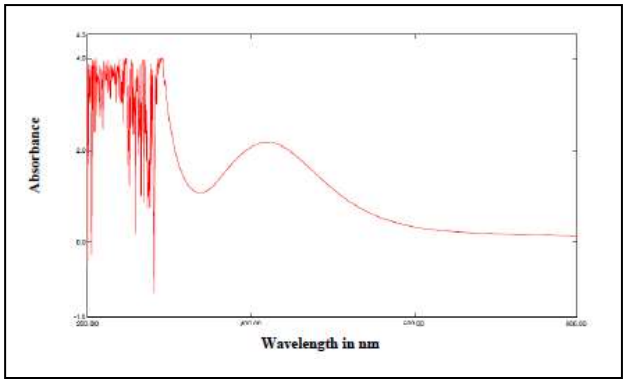

***Fig.7**. UV-Vis spectrum of the AgNP synthesized by 5mM $AgNO_3$ concentration*

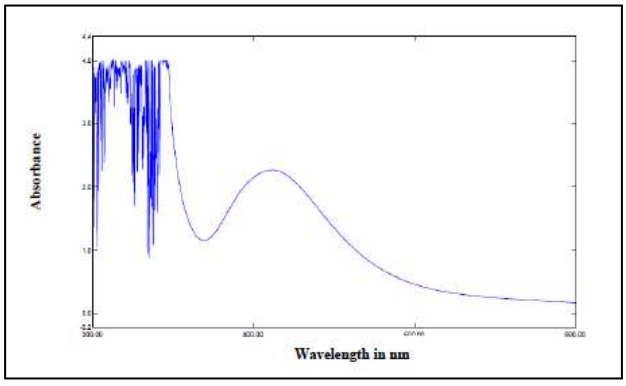

***Fig.8**. UV-Vis spectrum of the AgNP synthesized by 10mM $AgNO_3$ concentration*

3.4.3 Effect of temperature on synthesis of AgNPs

The temperature effect on the synthesis of AgNPs was studied in the range from 10°C to 100°C. It was observed that with increase in temperature there was an increase in the UV-visible absorbance spectrum. The highest yield of AgNPs was observed at 100°C (Fig. 9). The optimal temperature for sufficient yield of AgNPs was considered to be 40°C for practical purpose. It was observed that with the increase in temperature there was an increase in AgNP synthesis. The broadening of absorbance spectrum at low temperature indicates the synthesis of large sized AgNPs and the narrow absorbance spectrum at higher temperatures indicates the synthesis of small sized AgNPs which may be due to rapid reaction rate. The reduction of $AgNO_3$ was increased with increase in temperature which was indicated by the rapid color change of the solution [14].

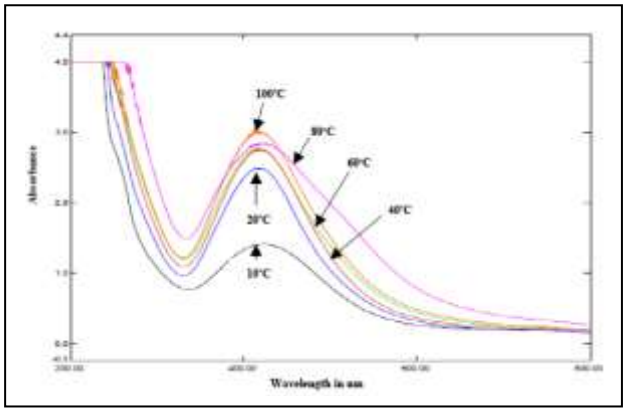

Fig.9*.UV- Vis spectrum of the AgNP synthesized at different temperatures.*

3.4.4 Effect of pH on biosynthesis of AgNPs

The effect of pH on the synthesis of AgNPs was studied for three different pH (5, 7 & 9), which is depicted in Fig. 10, 11 & 12. With increase in pH the absorption increased and gave the narrow peak at pH 9 with uniform distribution in the size of AgNPs. This result infers that the basic pH is favorable for synthesis of AgNPs. The size of AgNPs were affected by pH as it has the capacity to modify the charge of biomolecules, which in turn influence their capping and stabilizing properties [15].

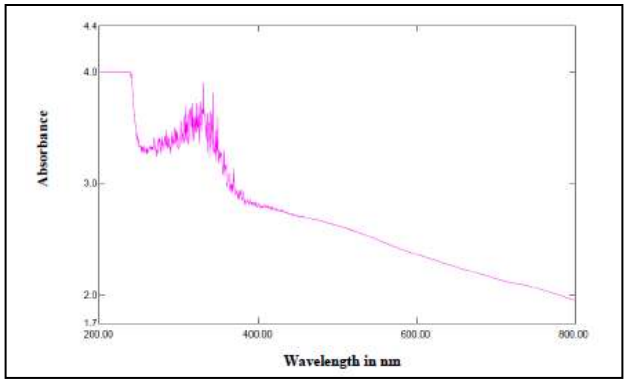

Fig.10*. UV-Vis spectrum of the AgNP synthesized at pH 5*

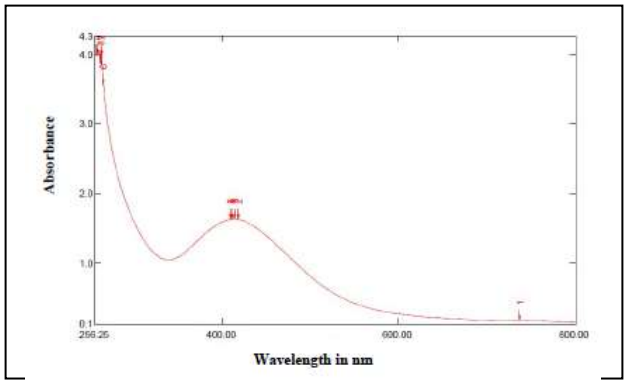

Fig.11*. UV-Vis spectrum of the AgNP synthesized at pH 7*

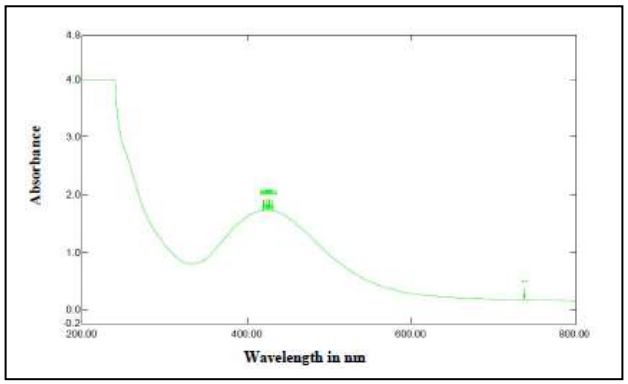

Fig.12*. UV- Vis spectrum of the AgNP synthesized at pH 9*

3.4.5 Effect of incubation period on biosynthesis of AgNPs

The reaction time plays a vital role in the AgNP synthesis. The influence of reaction time on the AgNP synthesis was studied at various incubation periods (120, 180 and 240 minutes) at ambient temperature. The leaf extract was added to $AgNO_3$ solution, which resulted in the color change from light yellow to brown within a span of 10 minutes. Fig. 13, 14 & 15 showed different UV–Vis spectrum for different time intervals. The color intensity was enhanced with increase in reaction time which may be because of conversion of large amount of Ag+ to Ag^o [16].

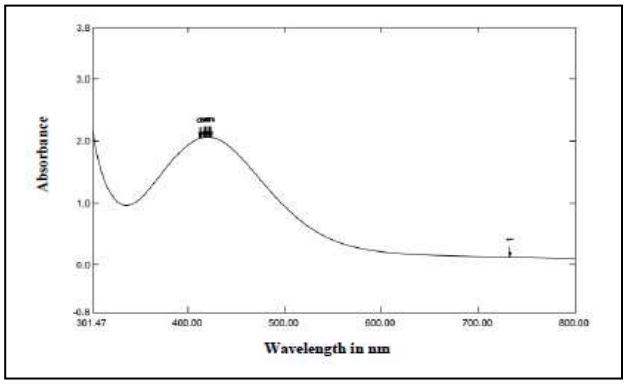

Fig.13. *UV- Vis spectrum of the AgNP synthesized at incubation period of 120minutes.*

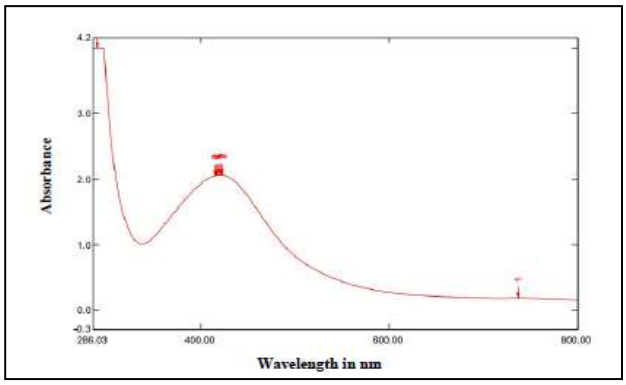

***Fig.14**. UV- Vis spectrum of the AgNP synthesized at incubation period of 180minutes.*

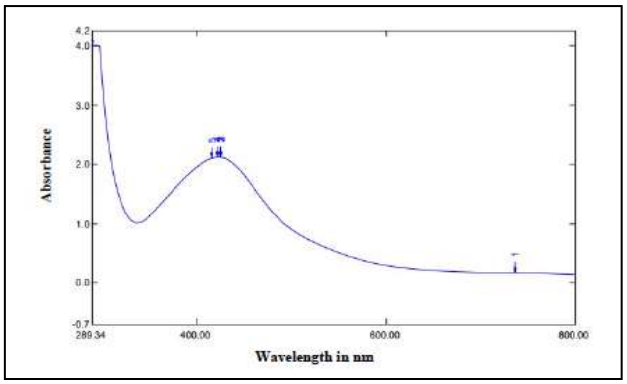

***Fig.15**. UV- Vis spectrum of the AgNP synthesized at incubation period of 240minutes.*

3.5 Applications of AgNPs

3.5.1 Antibacterial activity of silver nanoparticles

Application of synthesized AgNPs was studied by conducting antibacterial activity, antioxidant activity (DPPH and H_2O_2 assay) and detection of H_2O_2.

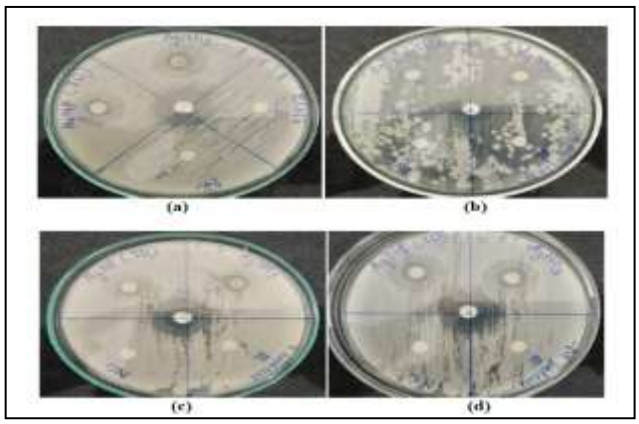

***Fig.16**. Antibacterial activity of silver nanoparticles on Gram negative bacteria (a) E. coli and (b) P.aeruginosa and Gram positive bacteria (c) S.aureus and (d) M.luteus*

***Table 2.** Zone of inhibition of AgNPs on Gram positive and Gram negative bacteria*

Bacteria	Mean diameter of Zone of inhibition (in mm)
Escherichia coli	12
Pseudomonas aeruginosa	18
Staphylococcus aureus	10
Micrococcus luteus	14

The different species of bacteria showed inhibition zone in the disk diffusion method of antimicrobial activity. Synthesized AgNPs showed antibacterial activity against both Gram positive bacteria and Gram negative bacteria as shown in Fig. 16. Gram negative bacteria Pseudomonas aeruginosa showed the maximum inhibition zone. The antibacterial activity may be because of ionic binding of the AgNPs on the bacterial surface creating great intensity of the proton motive force [17, 18].

3.5.2 Antioxidant effect of silver nanoparticles

DPPH and H_2O_2 assay were performed to determine the antioxidant effect of synthesized AgNPs.

DPPH antioxidant activity

The DPPH antioxidant activity was performed for various concentrations of leaf extract, AgNP concentrations and ascorbic acid ranging from 20µg/ml to 100µg/ml. The antioxidant activity of plant extract and AgNPs increased with the increase in the concentration of samples as shown in Fig.18. The antioxidant property of AgNPs was observed to be considerably significant in comparison to leaf extract and ascorbic acid.

***Table 3**. DPPH antioxidant activity*

Concentration in µg/ml	%DPPH antioxidant activity		
	AgNPs	Leaf extract	Ascorbic acid
20	35	37	56
40	44	41	61
60	58	53	70
80	69	65	81
100	83	78	84

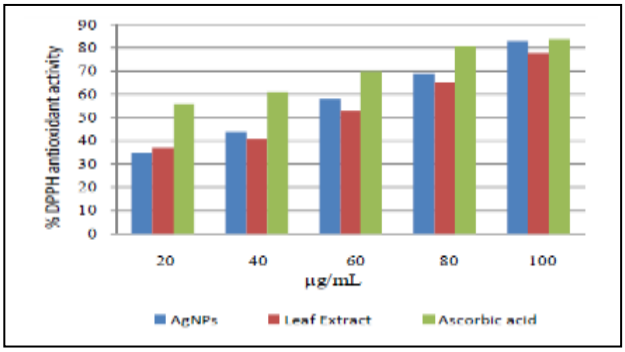

***Fig.17**. % DPPH antioxidant activity of silver nanoparticles*

H_2O_2 antioxidant activity

The H_2O_2 antioxidant activity was performed for various concentrations of leaf extract, AgNP concentration and ascorbic acid ranging from 20µg/ml to 100µg/ml. The

antioxidant property of plant extract and AgNPs increased with the increase in their concentration as shown in Fig.19. The antioxidant property of AgNPs was found to be considerably significant in comparison to leaf extract and ascorbic acid. The antioxidant activity of AgNPs may be due to phytochemicals such as phenolic compounds that are present in the leaf extract [19].

Table 4. *H_2O_2 antioxidant activity*

Concentration in µg/ml	% H_2O_2 antioxidant activity		
	AgNPs	Leaf extract	Ascorbic acid
20	30	38	41
40	51	56	60
60	72	71	77
80	78	76	81
100	89	85	92

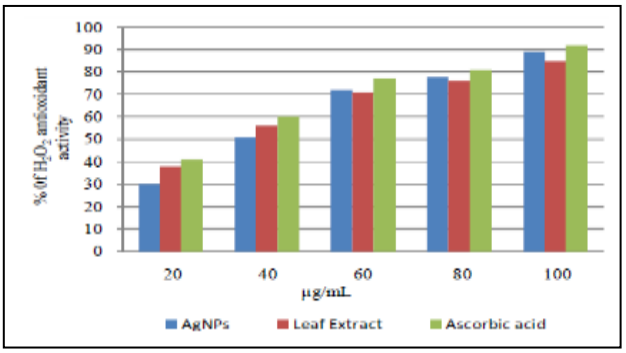

Fig.18*. % H_2O_2 antioxidant activity of AgNPs*

3.5.3 Detection of H_2O_2

Detection of H_2O_2 was performed by the addition of various concentrations of hydrogen peroxide to the synthesized AgNPs. The brown colored AgNPs decolorized after the addition of H_2O_2 and thus the presence of hydrogen peroxide was analysed by UV-visible spectrum. The least measurable concentration of H_2O_2 was determined by adding various concentrations of H_2O_2 to the known concentration of AgNPs. The value of the absorbance decreased with increase in concentration of H_2O_2 and finally disappeared at 100mM concentration, as revealed in Fig.19. Hence it can be inferred that the minimum detectable H_2O_2 concentration is 100mM. It was observed that the color of nanoparticles suspension changed from light brown to colorless signifying the oxidation of AgNPs. The mechanism involved in detecting hydrogen peroxide by silver nanoparticles may be due to high oxidizing ability of H_2O_2 [20].

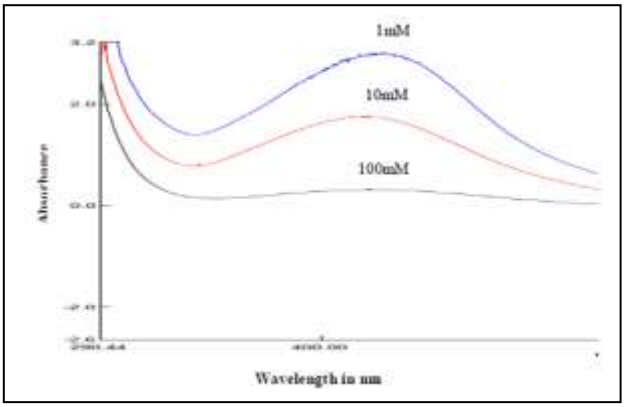

Fig.19*. UV- Vis spectrum of the AgNPs oxidized by H_2O_2*

4 CONCLUSIONS

A simple and economic approach has been attempted to obtain a green eco-friendly synthesis of AgNPs which was obtained from bio-reduction of $AgNO_3$ solution by leaf extract of T. procumbens. Synthesized AgNPs from the plant extracts were analysed using UV-Visible spectroscopy. The optimum conditions for synthesis of AgNPs from Tridax procumbens were found to be 1.0ml of leaf extract, 10mM concentration of silver nitrate, pH of 9, incubation time of 180minutes and temperature of 40^o C. Synthesized AgNPs showed good antibacterial property against all Gram positive and Gram negative bacteria. The highest inhibition zone was observed for Gram negative bacterium Pseudomonas aeruginosa. The synthesized AgNPs showed excellent antioxidant activity which was confirmed by DPPH and H_2O_2 assay. Further the application of AgNPs in the qualitative detection of H_2O_2 was studied.

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REFERENCES

[1] Geoprincy,G. A review on green synthesis of silver nanoparticles. Asian J.Pharm. Clin. Res. 6 (1) (2013) 8–12.

[2] Iravani S, Green synthesis of metal nanoparticles using plants, Green Chem. 13(2011) 2638–2650.

[3] Shet A R, Shwetha T, Arvind B. Economical biosynthesis of silver nanoparticles using fruit waste. Journal of chemical and pharmaceutical sciences. 9(3) (2016), 2306-2311.

[4] Akshay R, Elizabeth G, Akshay S, and Jameel S. Optimization of Green Synthesis of Silver Nanoparticles from Leaf Extracts of Pimenta dioica (Allspice). The Scientific World Journal.12(4) (2013), 1-5. Article ID 362890, http://dx.doi.org/10.1155/2013/362890.

[5] Henry F, Harry K, Audy D. Synthesis of Silver Nanoparticles Using Aqueous Extract of Medicinal Plants (Impatiens balsamina and Lantana camara) Fresh Leaves and Analysis of Antimicrobial Activity. International Journal of Microbiology.2019, Article ID 8642303, 1-8. https://doi.org/10.1155/2019/8642303

[6] Logeswari, P. Silambarasan, S. Abraham J. "Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property", Journal of Saudi Chemical Society. 12(19).(2015). 311-317.

[7] Catalina Q, Natalia A, Jenniffer Z, Luz E, Botero, J, Diana Z, Jorge S,Vera Z. Optimization of silver nanoparticle synthesis by chemical reduction and evaluation of its antimicrobial and toxic activity. Biomaterials Research. 8(2) (2019). 23-27. https://doi.org/10.1186/s40824-019-0173-y

[8] Amrita Singh, Brijesh G,Sandesh. J. Optimization of synthesis parameters of silver nanoparticles and its antimicrobial activity. Materials Science for Energy Technologies. 3 (2020) 232–236.

[9] Dhanalakshmia T, Rajendran S, Synthesis of silver nanoparticles using Tridax procumbens and its

antimicrobial activity. Scholars Research Library Archives of Applied Science Research. 4 (3) (2012), 1289-1293.
[10] Rajaram S, Sawant, Ashvin G,Godghate. Preliminary Phytochemical Analysis of Leaves of Tridax Procumbens Linn. International Journal of Science, Environment and Technology. 3(2). (2013) 388 –394.
[11] Mudara K, Chinthika P, Pradeep M, Nuwan D,Arachchi,Neluka F. Biosynthesized silver nanoparticles: are they effective antimicrobials?. Mem Inst Oswaldo Cruz, Rio de Janeiro. 112(8), (2017) 537-543.
[12] Ram Prasad, Vyshnava S. Antibacterial Activity of Silver Nanoparticles Synthesized by Bark Extract of Syzygium cumini. 2013, 1-6. Article ID: 431218, http://dx.doi.org/10.1155/2013/431218
[13] Salomoni R, Léo P, Montemor AF, Rinaldi BG, Rodrigues M. Antibacterial effect of silver nanoparticles in Pseudomonas aeruginosa. Nanotechnology, Science and Applications. 10 (3) (2017), 115–12.
[14] Krishnaraj C,Ramachandran R, Mohan K, Kalaichelvan P, Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi, Spectrochim. Acta Part A: Mol. Biomol.Spectrosc. 93 (2012) 95–99.
[15] Chung I,Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G. “Plant-mediated synthesis of silver nanoparticles: Their characteristic properties and therapeutic applications”, Nanoscale Research Letters. 3(11).(2016). 40-54.
[16] Veerasamy R, Ravichandran V, Tiah Z , Subashini G , Terence F , Eddy F , Nelson J, Sokkalingam A. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities, J. Saudi Chem. Soc. 15(2) (2011) 113–120.
[17] Shet A R, Pritam G, Laxmikant P,Veeranna H. A preliminary study on green synthesis and antibacterial activity of silver nanoparticles. International Journal of current Biotechnology. 3(2) (2015), 1-6.
[18] Ramya M. Sylvia S. Green synthesis of silver nanoparticles, Int. J. Pharm. Med.Biosci. 1 (1) (2012) 54–60.
[19] Bhakya S. Muthukrishnan M. Sukumaran M. Muthukumar Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity Appl Nanosci. 6(5) (2016), 755–766. DOI 10.1007/s13204-015-0473-z
[20] Kaushik R, Chandan K. Sarkar, Chandan K. Ghosh. Fast colourimetric detection of H_2O_2 by biogenic silver nanoparticles synthesised using Benincasa hispida fruit extract Nanotechnol Rev. 5(2) (2016) 251–258.