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PHYSICOCHEMICAL CHARACTERIZATION AND ANALYSIS OF POTENTIAL ANTIBACTERIAL PROPERTY OF BIOGENIC SILVER NANOPARICLES OF LEAF AQUEOUS EXTRACT OF *Tridax procumbens*

TEJOVATHI G[1*], ZAMAN MB [#2] AND PRAGYA S[3]

1: Department of Life Science, Vijayraje Institute of Science and Management, NH-75, Turari, Gwalior 475001,

2[#]: School of Studies in Physics, Jiwaji University, Gwalior 474011

3: Department of life science, Vijayraje Institute of Science and Management, NH-75, Turari, Gwalior 475001

[*]**Corresponding Author: E Mail: teja_biotech@vismgwalior.com; Phone: 9039646493**

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ABSTRACT

In the present study, silver nanoparticles (AgNPs) were synthesized using leaf aqueous extract of *Tridax procumbens* prepared by distillation in soxhlet apparatus (sample-1) and by boiling in microwave oven (sample2). Development of nanoparticles (NPs) was noticed through colour change in the solution from pale brown to dark blackish brown. The physic chemical characters such as optical density analysis, phase analysis and functional groups determination were observed.

UV Vis spectrophotometer analysis of optical density has shown broad peak at 438nm in sample 2 and at lower wave length (430nm) in sample 1. X-ray diffraction (XRD) studies recorded much intense and sharper peaks in sample 2 than in sample 1, confirming the highly crystalline nature of NPs in sample 2. The average crystal size was found to be approximately 16nm and 30nm in sample 1 and 2 respectively. FTIR spectral analysis for functional groups present in NPs indicates the presence of aliphatic compounds, olefin, hydroxyl and aliphatic nitro compounds.

In vitro sensitivity studies by agar well diffusion method have demonstrated good antibacterial property of AgNPs against *E. coli*, *S. typhi* and *L. acidophilus*.

Keywords: *Tridax procumbence*, Silver nanoparticles, antibacterial, XRD, FTIR, UV Vis-spectroscopy

INTRODUCTION

Metallic nanoparticles are ultrafine particles with size less than 100nm (Kato, 2011). The high surface to volume ratio of these particles has lead to enhanced physical and chemical properties and resulted in greater applications in the field such as medicine, biotechnology (Bhutt, 2003). Though several physical and chemical methods are available for the nanoparticles (NPs) synthesis, biogenic synthesis is the safest, eco-friendly, fast and low cost technique, hence is an attractive approach for nanoparticles synthesis (Devendra *et al*., 2009; Narayanamma *et al*., 2016). Among the various metal nanoparticles developed so far, silver nanoparticles (AgNPs) have gained tremendous attention because of their unique properties such as chemical stability, good conductivity, catalytic action, and great applications in medicine and other fields (Sundarrajan *et al*. 2017, Ravindran *et al*., 2016).

During the green synthesis of NPs various biomolecules such as proteins, carbohydrates, phenols, saponins, tannins, terpenoids, vitamins etc., present in the plant extracts are reported to reduce, cap and stabilize metallic ions (Ajitha *et. al*., 2014, Rao *et al*. 2014, Elumalai . 2014).

Tridax procumbens L, a perennial creeping herb that grows as weed in tropical, subtropical, mild temperate regions in world wide is commonly also known as ‘Ghamra’ and ‘Coat button’. The plant is rich in ketones, phenols, alkines, amines and lactones (Khan *et al*., 2008; Ankita and Jain, 2012, Kale and Deshmukh, 2014).

Tridax is extensively reported to be used in Indian traditional medicine for wound healing, blood clotting, diarrhea, dysentery etc. (Ravikumar *et al*., 2005a,b). Plant extract has been reported in folk medicine for the treatment of skin infections (Taddei and Roses-Romero, 2000). Tridax is best known as Bhringraj, for curing liver disorders in Ayurveda (Kuchekar *et al*., 2018). Few reports on nanoparticles development using various metals and extracts of different parts of Tridax are available (Senthil and Ramesh , 2012; Himakshi and Malik, 2014; Kuchekar *et al*., 2018; Manokari and Shekhawat, 2017; Kalpana *et. al*., 2016;). Present study reports

the role of extraction method on the development of NPs, their physic chemical properties and on their *in vitro* antimicrobial property.

MATERIAL AND METHODS

The fresh leaves of Tridax collected from the college campus, were washed with running tap water and then shade dried. The aqueous leaf extract was prepared by two methods - 1. in Soxhlet (sample 1) and 2. In Microwave (sample 2). About 5 grams of dried leaf powder was distilled in soxhlet apparatus in 200ml of distilled water (sample1). Another sample was prepared by boiling in microwave for 10 minutes. Thereafter the extract was cooled and filtered with Whatman No. 1 paper (Sample 2). Both the extracts were stored in the refrigerator for further use.

Preparation of Silver nanoparticles

0.1M silver nitrate solution was mixed with aqueous leaf extract (25 ml) and left in the dark for over night.

Physicochemical analysis

The AgNPs were collected by centrifugation at 10,000rpm for 10minutes and then washed then thrice with sterile distilled water by centrifugation.

UV-Vis spectra analysis

The reduction of silver nitrate solution and formation of silver nanoparticles (AgNPs) was analyzed by recording optical density. About 100µg of NPs from sample 1 and 2 were suspended in 1ml distilled water and were scanned between 300nm – 900nm wavelength using Shimadzu UV-2450 model spectrophotometer.

XRD analysis

Approximately 80 µg dried samples of 1 &2 were scanned for phase identification using the instrument-X-ray Diffractometer (Rikagu Miniflex 600 model).

The average crystallite size was calculated using the Scherrer relation:

$D= 0.9 \lambda / \beta \cos\theta$

Where D is crystallite size, λ the wavelength and β is full width at half maximum.

Fourier Transform Infrared spectroscopy (FTIR) analysis

Perkin Elmer IR spectrometer instrument was used to determine the functional groups of the AgNPs, in sample 1 and 2, between 500 cm^{-1} to 4000 cm^{-1} range.

Optical, XRD and FTIR studies were carried out at Central Instrumentation Facilities at Jiwaji University, Gwalior.

In vitro antibacterial studies

According to the procedure given by Pratibha *et al*., (2017), antimicrobial activity of AgNPs was determined by *in vitro* sensitivity analysis against *S. typhi, L. acidophilus* and *E. coli*, using Agar well diffusion method

(Okeke *et al*., 2001). Gentamycin was used as a standard drug and its minimum inhibition concentration (MIC) against each bacterial strain was detected using 'Ezy MIC strips' (Hi media) with concentration gradient of 0.04 to 1024mcg/ml.

The oven dried Tridax leaf aqueous extract, dissolved in 5ml distilled water, was used at 20µl concentration as control for antimicrobial analysis.

Sterilized Muller Hinton Agar (MHA) medium was used for *in vitro* sensitivity test. Bacterial cultures were collected using a sterile swab and spread plated (Kirbybaur method) on a sterile MHA plate to achieve a confluent growth. Then wells were created using 5mm well borer at equal distance in the medium. The *Tridax* leaf extract and AgNPs soln., each at 20µl concentration were poured into the separate wells. The plates were incubated overnight at 37^0C.

The zone of inhibition around the wells was recorded in mm (diameter) for each bacterial strain against leaf extract and AgNPs.

RESULTS AND DISCUSSION

Change in the color of the solution is the primary indication for the bioreduction of silver ions and formation of silver nanoparticles (AgNPs). A gradual shift in the color of the samples (1 & 2) from light brown to dark blackish brown color was observed in the solutions on mixing of extract with $AgNO_3$ solution (Figure 1a,b). The samples were centrifuged and the precipitate was dried (Figure 1c).

In order to confirm the formation of silver nanoparticles, the samples optical density was monitored at 300nm to 800nm wavelength using UV-VIS absorption Spectrometer. Metallic nanoparticles were reported to show surface Plasmon resonance absorption at UV Vis spectrophotometric region (Padmaja *et al*., 2011).

Figure 2a shows the absorption spectra of sample 1 and sample 2. A broad peak was observed at 430nm and 438nm with sample 1 and 2 respectively. The absorption peak in sample 1 was slightly at a lower wavelength (430 nm) that indicates the blue shift in wavelength, which is ascribed to the size of the nanoparticles. Smaller the size of particles more will be the blue shift. Our finding are in accordance with earlier studies indicating the absorption peaks between 430nm-460nm for AgNPs (Erick and Padmanabhan, 2014; Rajeshkhrdy *et al*., 2010).

To find the phase and crystallinity of the synthesized samples, X-ray diffraction (XRD) tool was employed. The XRD results of AgNPs given in Figure 2b for the sample 1 and sample 2, clearly shows very sharp peaks

indicating that the NPs in the samples were highly crystalline. Peaks observed at two theta values of 38.34°, 46.32°, 55°, 57.56°, 64.7° and 67.66° were believed to be the reflections of (111), (103), (006), (105), (110) and (106) set of planes respectively. The peak at two theta value of 38.34° corresponds to the cubic phase which is much intense and sharper in Sample 2, confirming its highly crystalline nature than Sample1. The calculated crystallite size was found to be approx. 16 nm and 30 nm for sample 1 and sample 2 respectively.

The biomolecuels in the plant extracts are known to cap the silver metallic ion through their functional groups (Bar *et al*., 2009). The functional groups in the samples were characterized by FTIR analysis using Perkin Elmer IR spectrometer. Figure 3 shows the FTIR spectra for sample 1 and sample 2. The spectra of both samples were similar and showing peaks at 3426 cm^{-1}, 2926 cm^{-1}, 1627 cm^{-1}, 1386 cm^{-1} and 1110 cm^{-1}, which correspond to presence of aliphatic compounds, olefinic groups, hydroxyl groups and aliphatic nitro compounds. Absorption peak around 3426 cm^{-1}occurs due to normal polymeric O-H stretch denotes alcohol or phenol, whereas peak at 2926 $cm^{-1}$is due to C-H stretch of methylene group. The absorption peak at 1627 cm^{-1} can be assigned to C=C stretch and the peak at around 1380 cm^{-1} confirms the presence of nitrate ions and halogen compounds or secondary alcohols. The peaks of alcohol and phenol and C=C stretch observed in FTIR analysis of metal NPs in earlier reports were in accordance with the present results.

Antimicrobial activity analysis

Antimicrobial properties of metal nanoparticles are reported earlier with many plants (Erick and Padmanabhan, 2014; Dhanalakshmi and Rajendran, 2012; Gopalakrishnan *et al*., 2012; Sangeeta *et al*., 2016). Figure 4 shows the inhibition of *E. coli* growth to Gentamycine (3a), leaf extract (3b) and the AgNPs (3c).

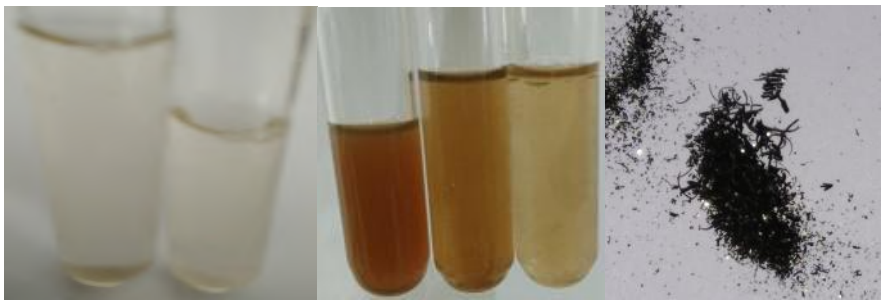

Figure 1: change in the colour of the silver nitrate and Tridax leaf extract mix solution a) initial b) after overnight incubation c) Purified dried nanoparticles

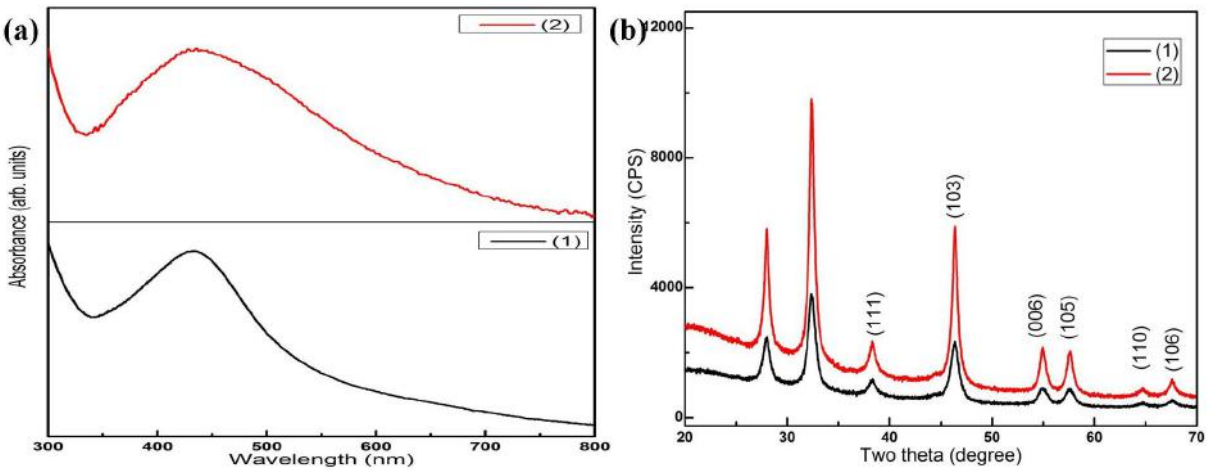

Fig. 2: (a) Absorption spectra of AgNPs developed from the Tridax leaf extract Sample 1and 2. (b) XRD spectra of AgNPs developed from the Tridax leaf extract Sample 1and 2

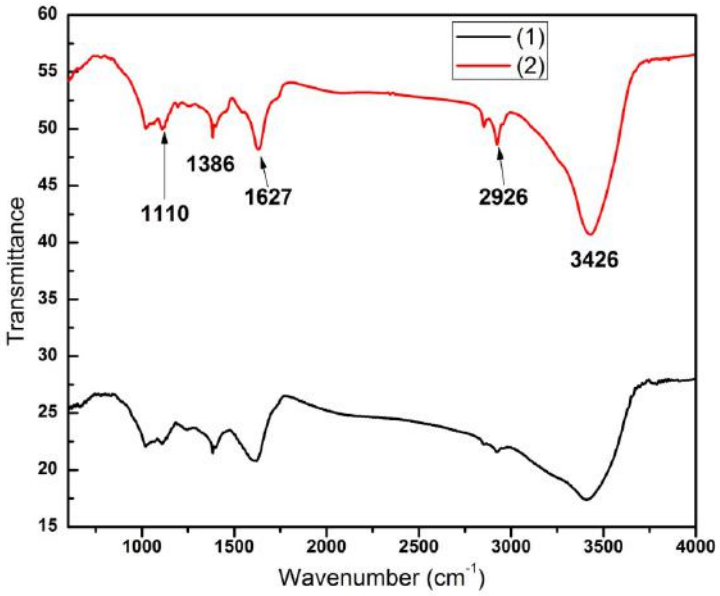

Fig. 3: FTIR spectra of AgNPs developed from the Tridax leaf extract Sample 1and 2

Figure 4: Zone of inhibition observed against (a) gentamycin (b) Tridax Leaf extract (c) Silver nanoparticles

The minimum inhibition concentration (MIC) of Gentamycin was recorded as 2mcg for *E. coli*, and S. *typhi*. While, MIC recorded for *L. acidophilus* was 0.51mcg. *In vitro* sensitivity test results of AgNPs against *E. coli, S. typhi* and *L. acidophilus*, the zone of inhibition was 7.0mm, 7.0mm and 9.5mm respectively. While the leaf extract has shown very less (5.1mm) inhibition zone indicating the effective antibacterial property of the synthesized *Tridax* AgNPs. Similar response was reported earlier too.

The present study concludes that pant extract preparation process holds important place in the types of metabolites and their concentration in the extracts. It also influences the plant molecules that cap the metal ions leading to nanoparticles synthesis, influence the physicochemical properties and other biological properties. Our results in this study with samples1 and 2 indicate that the microwave sample (2) appeared to be better than the sample 1.

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