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Biopotential of *Verbesina encelioides* (stem and leaf powders) in silver nanoparticle fabrication

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Abstract: Nanotechnology has opened new opportunities in the field of medical sciences and pharmaceuticals. Commercially, nanoparticles are in great demand in electronics, catalysis, chemistry, energy, and medicine. Today in nanoparticle production green synthesis, using biological material including medicinal plants as a starting material, is in vogue. In the present investigation the powder extract (stems and leaves) of an important medicinal plant, *Verbesina encelioides*, was used for the biosynthesis of bionanoparticles. The synthesized nanoparticles were screened and characterized by UV-visible spectrophotometer, scanning electron microscopy, X-ray diffraction, and FTIR analyses. The particles were then subjected to antimicrobial assay. Comparative analyses of the antimicrobial behavior of aqueous, ethanolic, and methanolic extracts and bionanoparticles against 2 bacteria (*Escherichia coli* and *Vibrio cholerae*) and 2 fungi (*Aspergillus niger* and *A. flavus*) are described.

Key words: Nanoparticles, green synthesis, UV-visible spectrophotometer, scanning electron microscopy, X-ray diffraction, FTIR, antimicrobial assay

1. Introduction

Nanotechnology deals with the production, manipulation, and use of material that ranges from less than a micron in size. A nanometer is 1 billionth of a meter or roughly the length of 3 atoms side by side (Kannan and Subbalaxmi, 2010). Bionanotechnology on the whole deals with the interaction of biology, chemistry, material sciences, engineering, and medicine. Silver nanoparticles are of immense use due to their size, catalytic properties, and antimicrobial behavior (Reda et al., 2011). The green synthesis of nanoparticles, on the other hand, concerns the transformation of biological systems to create new devices on a nanoscale. These are produced through various physical and chemical processes (Parashar et al., 2009); however, all of these methods have detrimental effects due to increased size, high energy and capital investment requirements, and the use of toxic chemicals (Joerger et al., 2000; Jain et al., 2009). Keeping in view the disadvantages entailed in both processes, we have adopted biogenic methods for the synthesis of bionanoparticles. These methods are clean and less expensive, require less energy, and employ ambient conditions (Saxena et al., 2010). Biogenic methods have slow kinetics and allow for better manipulation and control over crystal growth and stabilization. In recent years there has been an upsurge in

green synthesis routes that permit better shape and size control for various nanotechnological applications. The use of eco-friendly materials such as plant extracts (Jain et al., 2009), bacteria (Saifuddin et al., 2009), fungi (Bhainsa and D'Souza, 2006), and enzymes (Willner et al., 2006) for the synthesis of bionanoparticles offers innumerable benefits for pharmaceutical and general biomedical applications (Kowshik et al., 2002; Vdayasoorian et al., 2011). Biogenic nanoparticles have a wide range of applications due to their catalytic properties (Jana et al., 1999), antimicrobial behavior (Pal et al., 2007), and biosensing and diagnostics (Schultz et al., 2000; Songping and Shuyuan, 2005). Moreover, bionanoparticles also exhibit antiproliferative activity against cancerous cells. A recent report demonstrated the interaction between bionanoparticles and HIV-I (Elechiguerra et al., 2005). Bionanoparticles using the dried leaf extract of *Boswellia ovalifoliolata* (Ankanna et al., 2010), *Cinnamomum camphora* (Huang et al., 2007), and *Pongamia pinnata* (Raut et al., 2010) have also been reported. Some recent studies have demonstrated that weeds having important medicinal properties (e.g., *Ipomoea aquatica*, *Enhydra fluctuans*, and *Ludwigia adscendens*) could also be utilized for the synthesis of bionanoparticles because of their ready availability and drought resistance (Roy and Barik, 2010).

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Verbesina encelioides contains flavonol, glycosides, primary metabolites, and triterpenoids and has immense pharmacological properties. Presumably it would possess antimicrobial, antiviral, antitumor, hypoglycemic, and antiimplantation efficacies. In a recent study, we reported the synthesis and functionality of biogenic nanoparticles using fresh extract of *V. encelioides* (Kushwaha and Malik, 2012).

The present study describes the use of *V. encelioides* powdered extract for reducing Ag^+ ions to synthesize bionanoparticles, which were then subjected to different physical analyses (UV-visible spectrophotometric analysis, scanning electron microscopy (SEM) analysis, X-ray diffraction (XRD), and FTIR analysis) to screen and characterize them. Samples were also subjected to antimicrobial assays to examine their potential against microorganisms.

2. Materials and methods

2.1. Preparation of powdered extracts (stems and leaves)

Branches (stems and leaves) of *V. encelioides* were collected from the campus of Jaipur National University, Jaipur (Rajasthan), India, and were thoroughly washed with running water for 15–20 min. They were placed in a hot air oven for drying at 70 °C and 78 °C overnight for 4–5 days, respectively. These dried specimens were ground into a fine powder with a mixer grinder. The resulting powders were stored for further use.

For the preparation of extracts, 25 g of the individual powders were weighed and mixed with 100 mL of triple deionized water. This mixture was boiled for 15 min on a hot plate and filtered using Whatman filter paper no. 1.

2.2. Preparation of silver nitrate solution and reaction mixture

Aqueous silver nitrate solutions (5 mM and 10 mM) were prepared using silver nitrate powder and triple deionized water in a fixed ratio. The reaction mixture was prepared by taking 10 mL of the prepared filtrates obtained from the plant and 90 mL of silver nitrate solution. This reaction mixture was prepared with each molar concentration of silver nitrate solution and incubated at room temperature for up to 5 h. Observations were made after each hour of incubation to observe changes in color ranging from light greenish to dark brownish. After each hour, a small amount of the reaction mixture was centrifuged at 18,000 rpm for 25 min, the supernatant was collected, and the pellet was stored at 4 °C. The collected supernatant was heated at 50–90 °C and the change in color, indicating the formation of bionanoparticles in the reaction mixture, was observed.

2.3. UV-visible spectroscopy

Synthesized bionanoparticles were characterized by UV-Vis spectroscopy, which is one of the most widely

used techniques for structural characterization of silver nanoparticles (Sun et al., 2001). The bioreduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction mixture 1–5 h after diluting a small amount of the sample in triple deionized water. A Genesys 10 UV spectrophotometer was used to screen the reducing power of the Ag^+ synthesized in the reaction mixture. This analysis was performed using the silver nitrate solution for stem (10 mM) and leaf (5 mM), and the absorbance was recorded from 300 nm to 600 nm.

2.4. SEM analysis

SEM analysis was performed for further characterization of the samples (shape and size). For this analysis a ZEISS EVO-50 INCA Penta FET x3 scanning electron microscope and carbon coated stubs were used after the process of ultrasonication. Extra sample was removed from the stubs with blotting paper. The prepared stubs were air-dried and subjected to further analysis.

2.5. X-ray diffraction

This analysis confirmed the size of the bionanoparticles. A thin layer of the sample was prepared on a glass slide, and the preparation was subjected to further analysis with an ISO-DEBYEFLEX 2002 XRD machine using the Debye-Scherrer formula:

$$D = (0.94 \lambda) / (\beta \cos \theta),$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum, and θ is the diffraction angle.

2.6. FTIR analysis

FTIR analysis was done to identify the capping and stabilizing compounds responsible for the formation of bionanoparticles using a BRUKER-VERTEX-70 machine. OPUS software was used for sample analysis.

2.7. Antimicrobial assay

2.7.1. Antibacterial test

Antibacterial assays of bionanoparticles were performed with food poisoning and agar disk diffusion methods using the human pathogens *Escherichia coli* and *Vibrio cholerae*; antibiotics were used as the control.

For agar disk diffusion, 100 μL of freshly prepared culture inoculum was spread on petri plates containing Luria-Bertani medium. A sterile paper disk containing bionanoparticles along with 3 control disks were placed in the petri plate and incubated at 37 °C for 48 h, and observations were made. For *V. cholerae*, TCBS medium was used with 100 μL of fresh inoculum.

For the food poisoning method, bionanoparticles were added to EMB medium at the time of pouring. Solidified petri plates were streaked with *E. coli*; culture medium devoid of bionanoparticles was used as the control. For *V. cholerae*, TCBS medium was employed.

2.7.2. Antifungal test

The antifungal assay was performed using petri plates containing 15 mL of PDA medium supplemented with bionanoparticles. *Aspergillus flavus* MTCC 277 and *A. niger* were point-inoculated into the medium individually and incubated at 28 °C for 72 h. The diameter of the mycelial colony on the bionanoparticle-containing PDA plates was compared with the diameter of colony obtained on control plates (without bionanoparticles). The inhibition of fungal growth was calculated by the following formula:

$$\% \text{ inhibition} = (C - E) \times 100 / C,$$

where C = diameter of fungal mycelium on the control plate and E = diameter of fungal mycelium on the experimental plate.

3. Results

The reduction of silver ions into silver particles following exposure to plant extracts could be deciphered by a change in color. Bionanoparticles exhibited a dark yellowish color initially but changed to brown due to the surface plasmon resonance phenomenon. The change in color of the reaction mixture positively indicated the formation of bionanoparticles. The indication test was used to confirm the synthesis of bionanoparticles in the aqueous solution. In this test the supernatants obtained during the centrifugation process were collected individually for stem and leaf extracts and heated at 50–90 °C. The change in color of the supernatant during heating was a positive indication for the synthesis of bionanoparticles (Figures 1 and 2). The results of the present investigation are interesting due to the identification of medicinal weeds as a potential source for

synthesizing bionanoparticles. The UV-Vis spectrograph of the colloidal solution of bionanoparticles was recorded. The technique outlined above appears to be very useful for the analysis of nanoparticles (Sastry et al., 1997, 1998).

During the UV-visible spectral analysis it was found that the formation of stem (powder) derived bionanoparticles was initiated during 1 h of incubation at room temperature, with the peak at 440 nm (Figure 3a). In leaf (powder) derived bionanoparticles a similar peak was observed after a 3 h incubation (Figure 3b).

The synthesized bionanoparticles were characterized using SEM analysis. Their size was revealed by applying 1500 kV of voltage and 30,000 KX magnification, keeping WD 8.0 mm for stem (Figure 4a); for leaf the magnification was 75,000 KX and WD 8.5 mm (Figure 4b).

XRD was performed to confirm the crystalline nature of the particles for stem and leaf bionanoparticles. The XRD pattern showed 2 intense peaks in the whole spectrum of 2θ values ranging from 10 to 60. The data indicated a greater potential for extracellular synthesis of bionanoparticles in the range of 11.38–36.22 nm with an average size of 23.8 nm from stem and 14.33–75.91 nm with an average size of 45.12 nm from leaf derived bionanoparticles.

Carbonyl groups from the amino acid residues and peptides of proteins show strong affinity for binding with metals (Lin et al., 2005), suggesting that the protein could act as an encapsulating agent and, hence, protect nanoparticles from agglomeration. Furthermore, the amide linkages in proteins and polypeptides are well characterized in the IR region. The FTIR spectra of our vacuum dried stem and leaf extracts showed absorption

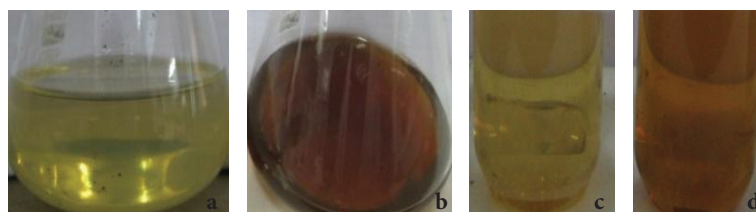


Figure 1. a) Color of the powdered stem extract reaction mixture at the time of mixing and b) color of the reaction mixture after incubation period of 5 h. c) Color of the supernatant after the process of centrifugation and d) change in color of the supernatant after boiling, indicating the formation of bionanoparticles in the reaction mixture.

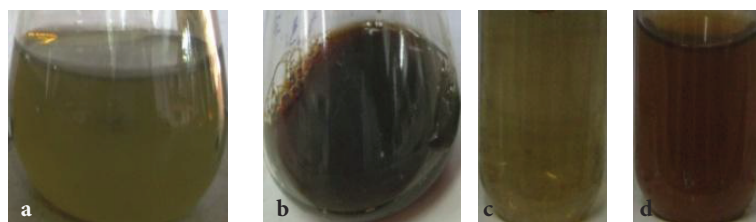


Figure 2. Results same as in Figure 1, but with powdered leaf extract.

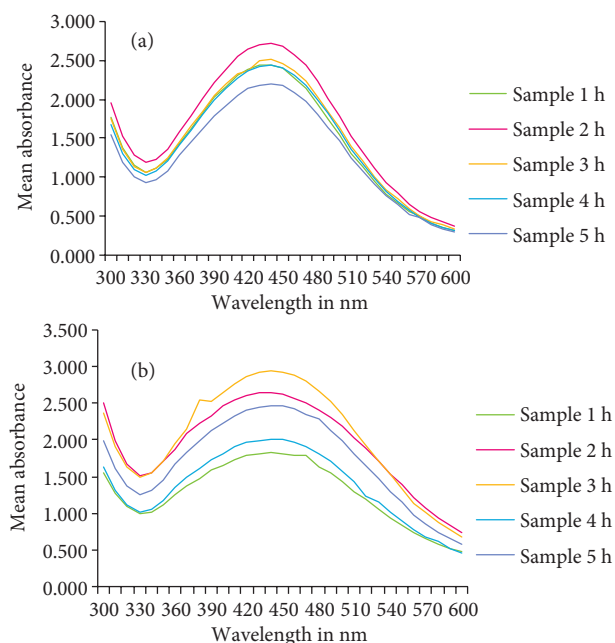


Figure 3. UV-visible spectral analysis of a) stem and b) leaf extracts using 10 mM and 5 mM of silver nitrate solution over 5 h, respectively.

bands in regions ranging from 3448.21 to 529.02 cm^{-1} and 3448.63 to 529.09 cm^{-1} , respectively (Table 1).

Effective bactericidal results were also obtained using *E. coli* and *V. cholerae* by disk diffusion method using 30 and 40 ppm of synthesized bionanoparticles, respectively (Figures 5a, 5b, 6a, and 6b). The results are given in Tables 2a and 2b.

In another experiment we performed an antimicrobial assay using *E. coli* food poisoning (Figures 7a–7c) and *V. cholerae* food poisoning (Figures 8–8c) methods. The data are given in Tables 3a and 3b.

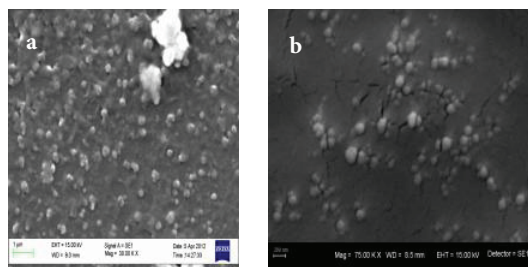


Figure 4. SEM analysis of a) stem and b) leaf extracts.

Synthesized bionanoparticles (50 ppm) indicated antifungal activity against *A. niger* and *A. flavus*. Zones of mycelia growth were measured to determine the percent inhibition, which was variable for stem and leaf bionanoparticles (Table 4; Figures 9a–9c and 10a–10c).

The results obtained led us to evaluate the antimicrobial activity of the plant extract and to compare it with the synthesized bionanoparticles using *E. coli* (Table 5; Figures 11a and 11b) and *V. cholerae* (Figures 12a and b).

4. Discussion

Currently, synthesis of nanoparticles is attracting added attention. Of the 3 routes employed, biological synthesis is preferred because it is cost-effective, and the ability to maintain homogeneity and stability of the synthesized nanoparticles is high.

Biological synthesis involves the use of microorganisms (Nair and Pradeep, 2002), enzymes, and plant and/or plant extracts (Shankar et al., 2004), and it is eco-friendly. Moreover, biological systems tend to provide a number of metal or metal containing particles in the nanometer range. Interestingly, both unicellular and multicellular organisms are reported to produce inorganic materials either intra- or extracellularly.

Table 1. FTIR spectral analysis of dried powders of stem and leaf derived Ag nanoparticles. Resultant groups were identified from the type of peak corresponding with a specific wave number (cm^{-1}).

Corresponding wave number (cm^{-1})	Type of peak	Resultant group
3448.21 and 3448.63	Broad	-OH stretching of alcohols and phenols
2882.03 and 2887.48	Medium	Aldehyde stretching of alkanes and primary amines
1467.77 and 1467.72	Medium	C-H (C-H_3 bend) of alkanes
1342.92 and 1342.84	Medium	Ketones (aromatic), nitro (conjugate or aliphatic) group
1280.84	Medium	C-N stretching vibration of amines or CO stretching of alcohols, ethers, carboxylic acid, esters, and anhydrides
1111.71 and 1110.62	Strong	Lactones
962.36 and 962.37	Medium	Alkanes
842.10 and 842.27	Medium	Aliphatic amines, alkanes

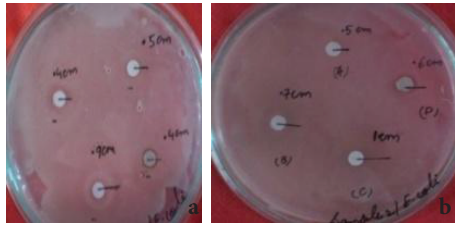


Figure 5. a) Showing different zones of inhibition using the control as (A) tetracycline, (B) penicillin, (C) streptomycin, and (D) stem extract synthesized bionanoparticle. b) Showing different zones of inhibition using the control as (A) tetracycline, (B) penicillin, (C) streptomycin, and (D) leaf extract synthesized bionanoparticle.

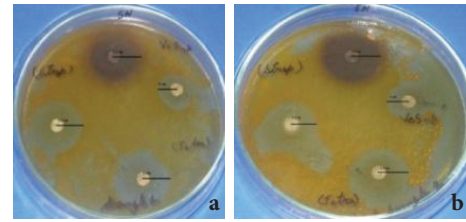


Figure 6. Showing different zones of inhibition using the control as Tetra (tetracycline) and Strep (streptomycin) and VeSnp (stem (a) and leaf (b) extract synthesized bionanoparticle) and SN (silver nitrate solution).

Table 2. Antibacterial test (*E. coli* and *V. cholerae* by agar disk diffusion method) using Ag nanoparticles of a) stem and b) leaf powdered extracts.

a) Data pertaining to stem powdered extract

Organism used	Treatment		Name of treatment	Zone of inhibition (mm)	Concentration used (ppm)
<i>E. coli</i>	A	Control	Tetracycline	4	30
	B		Penicillin	4	30
	C		Streptomycin	6	30
	D		Synthesized bionanoparticles	4	30
<i>V. cholerae</i>	Tetra	Control	Tetracycline	10	40
	Strep		Streptomycin	11	40
	SN		Silver nitrate	12	40
	VeSnp		Bionanoparticles	9	40

b) Data pertaining to leaf powdered extract

Organism used	Treatment		Name of treatment	Zone of inhibition (mm)	Concentration used (ppm)
<i>E. coli</i>	A	Control	Tetracycline	5	30
	B		Penicillin	7	30
	C		Streptomycin	10	30
	D		Synthesized bionanoparticles	6	30
<i>V. cholerae</i>	Tetra	Control	Tetracycline	14	40
	Strep		Streptomycin	12	40
	SN		Silver nitrate	14	40
	VeSnp		Bionanoparticles	10	40

In the current study we reported the synthesis of biogenic nanoparticles from the medicinally important plant *V. encelioides* using both leaf and stem extracts for the synthesis of biogenic nanoparticles. Due to the splitting of AgNO_3 into Ag^+ and NO_3^- , a change in the color of the reaction mixture was observed over time. Apparently the metabolites in the stem and leaf extracts acted as e^- donors

and reduced Ag^+ ions to Ag. Consequently, the formation of silver nanoparticles was indicated by the brown color of the aqueous solution following the excitation of surface plasmon vibrations (Shankar et al., 2004).

Our findings substantiate the data from *Capsicum annum* (Li et al., 2007) and *Aloe vera* extracts (Chandran et al., 2006); however, in the current study, we accomplished

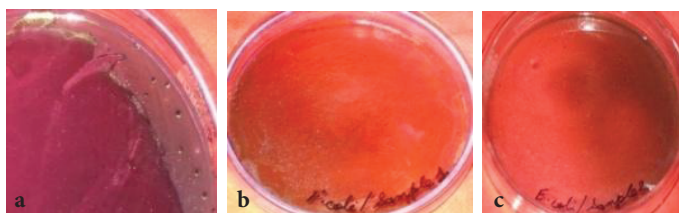


Figure 7. a) Control with *E. coli* growth; plates with the stem (b) and leaf (c) powder extract bionanoparticle with no growth of *E. coli*.

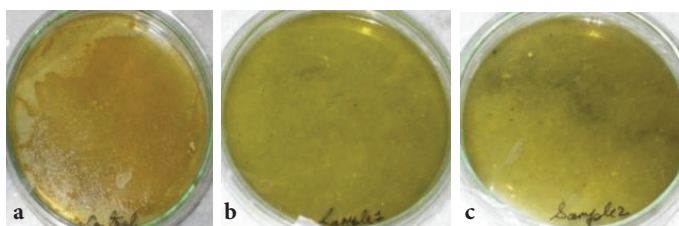


Figure 8. a) Control with yellow colonies on TCBS medium; plates with the stem (b) and leaf (c) extract bionanoparticles showing no growth.

Table 3. Bionanoparticles from a) stem powered extract and b) leaf powdered extract using food poisoning method.

a) Data pertaining to stem powdered extract

Organism	Disease caused	Concentration of synthesized bionanoparticles used	Control	Result
<i>E. coli</i>	Cholecystitis, bacteremia, cholangitis, diarrhea	30	Appearance of green metallic colonies	No growth was observed
<i>V. cholerae</i>	Cholera fever	40	Yellow colonies appear on TCBS media	No colonies were observed on TCBS media

b) Data pertaining to leaf powdered extract

Organism	Disease caused	Concentration of synthesized bionanoparticles used	Control	Result
<i>E. coli</i>	Cholecystitis, bacteremia, cholangitis, diarrhea	30	Appearance of green metallic colonies	No growth was observed
<i>V. cholerae</i>	Cholera fever	40	Yellow colonies appear on TCBS media	No colonies were observed on TCBS media

formation of nanoparticles at 5 mM and 10 mM of aqueous solution.

The reduction of silver ions during the incubation period is generally deciphered through UV-visible spectroscopy, and this period is variable, ranging from a few minutes to several hours (Mahitha et al., 2011). Further

characterization of synthesized biogenic nanoparticles is ascertained by transmission electron microscopy (TEM) (Renugadevi et al., 2012), SEM, and XRD. In our studies we have used all of the above mentioned techniques, except TEM, to characterize the synthesized biogenic nanoparticles. A review of the literature indicates that

Table 4. Antifungal assay (*Aspergillus niger* and *A. flavus*) using food poisoning method.

Sample used	Name of the species	Results (mycelia growth) (mm)		C Concentration (ppm)
Stem powder		Control	Sample	50
	<i>A. niger</i>	20	9	50
	<i>A. flavus</i>	10	6	50
Leaf powder	<i>A. niger</i>	20	9	50
	<i>A. flavus</i>	10	4	50

**Figure 9.** a) Fungal mycelia growth in control with no bionanoparticle sample; growth of fungal mycelia with stem (b) and leaf (c) extract bionanoparticle, respectively.**Figure 10.** a) Fungal mycelia growth in control with no bionanoparticle sample; growth of fungal mycelia with stem (b) and leaf (c) extract bionanoparticle sample, respectively.

the size of nanoparticles varies by species; for example, it was 8 nm in *Nicotiana* (Prasad et al., 2010), 26 nm in *Coriandrum* (Satyavathi et al., 2010), and from 5 to 30 nm in *C. quadrangularis*. In the current study of *V. encelioides*, we encountered sizes of 23.8 nm from stems and 45.12 nm from leaves.

Once the nanoparticles are produced they tend to agglomerate, and this largely depends on the chemistry as well as electromagnetic properties. To prevent agglomeration, the synthesized nanoparticles are coated with nonmagnetic substances to maintain their homogeneity. Different types of stabilizing agents have been used including PVC and thiourea including PEG (used in the present studies).

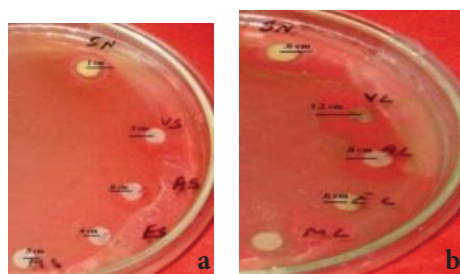
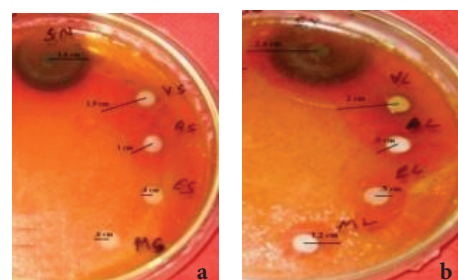
The XRD pattern of biogenic nanoparticles clearly illustrated that the synthesized nanoparticles were crystalline in nature. This confirms a report in *Nicotiana* (Prasad et al., 2010).

FTIR analysis monitors the capping agents responsible for the formation of nanoparticles. FTIR absorption spectra reveal absorbance bands in different regions. Thus, in *V. encelioides* 2882.03 and 2887.48 cm^{-1} indicated the presence of aldehyde stretching of alkanes and primary amines, 1342.92 and 1342.84 cm^{-1} the presence of ketones (aromatic) and the nitro (conjugate or aliphatic) group, 1111.71 and 1110.62 cm^{-1} the strong presence of lactones, and 842.10 and 842.27 cm^{-1} aliphatic amines and alkanes.

Interestingly, biogenic nanoparticles synthesized through the green route are pathogenic against the human pathogenic bacteria *E. coli* and *V. cholerae*. The toxicity was authenticated through food poisoning and disk diffusion methods. We used different concentrations (20, 30, 40, 50, and 60 ppm) of biogenic nanoparticles; among these, 30 and 40 ppm were recorded as the minimum inhibitory concentration for *E. coli* and *V. cholerae*, respectively. The zone of inhibition increased with the enhancement of

Table 5. Comparison of plant extracts with synthesized bionanoparticles (stem and leaf powders).

Organism used	Sample used	Plant extract				
		Aqueous extract (mm)	Ethanollic extract (mm)	Methanolic extract (mm)	Silver nitrate (mm)	Synthesized bionanoparticles (mm)
<i>E. coli</i>	Stem powder	8	6	5	10	9
	Leaf powder	8	6	No significant result	6	12
<i>V. cholerae</i>	Stem powder	10	4	6	16	19
	Leaf powder	9	5	12	24	20

**Figure 11.** Disk diffusion method results of stem (a) and leaf (b) powder derived bionanoparticles when compared with the aqueous stem extract (AS), ethanolic stem extract (ES), and methanolic stem extract (MS) along with silver nitrate (SN).**Figure 12.** Disk diffusion method results of stem (a) and leaf powder derived bionanoparticles when compared with the aqueous stem extract (AS), ethanolic stem extract (ES), and methanolic stem extract (MS) along with silver nitrate (SN).

biogenic nanoparticle concentration. Mahitha et al. (2011) reported the antibacterial effect of silver nanoparticles against gram-negative bacteria (*E. coli* and *K. pneumonia*), and we support this observation.

Ag ions and Ag salts have been used for decades as antimicrobial agents in divergent fields due to their growth-inhibitory capability against microorganisms. They have been used in the medical field for antimicrobial applications, especially to prevent HIV from binding to host cells (Nino-Martinez et al., 2008). In fact, silver nanoparticles and Ag-based compounds with antimicrobial effects are prepared through a variety of methods and are shown to have effective antimicrobial activity (Lee et al., 2008). When the 2 are compared, inorganic nanoparticles synthesized via the green route have a distinct advantage over conventional chemical antimicrobial agents.

It is suggested that the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, although the precise mechanism of the inhibitory effects on microorganisms is not clear. Some have suggested that the positive charge on the Ag ion is crucial for antibacterial activity due to electrostatic attraction between the negatively charged cell membrane of the microorganism and the positively charged nanoparticles (Dibrov et al., 2002). It has also been reported that concentration of Ag nanoparticles affects gram-negative bacteria through

the formation of pits in the cell wall (Sondi and Sondi, 2004). There is an accumulation of Ag nanoparticles in the bacterial membrane causing an alteration in permeability, which results in cell death.

According to Amro et al. (2000) cell death could be attributed to metal depletion, which results in the formation of irregularly shaped pits in the outer membrane. Consequently, membrane permeability is distorted due to the progressive release of lipopolysaccharide molecules and membrane proteins.

Recently, through the electron spin resonance study of Ag nanoparticles, Danilczuk et al. (2006) reported that the antimicrobial mechanism of Ag nanoparticles is somewhat associated with the formation of free radicals, which damage the membrane.

In addition, the free uptake of free silver ions may cause the disruption of ATP production and DNA replication (Lok et al., 2006).

It was also reported that synthesized biogenic nanoparticles exhibited antifungal effects, which were attributed to the destruction of membrane integrity (Kim et al., 2009) and inhibition of the normal budding process through cell membrane destruction (Praveen et al., 2012).

In the recent past, some workers have used different plant materials, e.g., leaves (Satyavani et al., 2011a, 2011b), fruits (Dubey et al., 2010), seeds (Bar et al., 2009b), latex

(Bar et al., 2009a), and bark (Sathishkuma et al., 2009). Leaf mediated synthesis of silver nanoparticles appears to be more profitable as it is simple and rapid (Daizy, 2011).

The present investigation demonstrated that powdered plant extracts were capable of producing Ag from an aqueous solution of Ag⁺. The method was a simple, quick, cost-effective, and environmentally friendly route for synthesizing bionanoparticles. Particles synthesized via the green route are highly toxic to multidrug resistant bacteria and, hence, have a great potential for biomedical and other applications including nanomedicines, nanoelectronics, and nano-optical devices. The green chemistry approach to the synthesis of bionanoparticles has many advantages; for example, it is easy to scale up and it is economically viable. Biologically synthesized nanoparticles could be of immense use in medical textiles due to their efficient antibacterial and antimicrobial properties. These eco-friendly nanoparticles are more acceptable for bactericidal, wound healing, and other medical applications due to their high selectivity, specificity, and sensitivity. They can

also aid in the early detection, diagnosis, and treatment of diseases, creating the potential for the large-scale synthesis of other inorganic materials (nanomaterials) (Tan et al., 2012). The antimicrobial functions of bionanoparticles pertinent to human pathogens involve a novel category of antibacterial agents. Presently, the synthesis of bionanoparticles can be confirmed by a change in color from yellowish to dark brown and quantitatively monitored by UV-Vis spectroscopy. The data emanating from SEM analyses revealed the average size of bionanoparticles as 23.8 nm for stem and 45.12 nm for leaf, respectively. It may be inferred that the stem is the most relevant source for the extracellular synthesis of bionanoparticles. This is the first report on synthesizing bionanoparticles using dried powders of stem and leaf obtained from *V. encelioides*.

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