

Original Article

DESMODIUM GANGETICUM ROOT AQUEOUS EXTRACT MEDIATED SYNTHESIS OF NI NANOPARTICLE AND ITS BIOLOGICAL EVALUATION

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ABSTRACT

Objective: A novel, eco-friendly method of Nickel nanoparticles synthesis using aqueous extract of *Desmodium gangeticum* root (DG) has been reported in this study.

Methods: Novel approach on synthesis of Ni nanoparticles using NiCl₂ as precursor and aqueous extract of *Desmodium gangeticum* root as the reducing agent. Nanoparticles were characterized for its average size, morphology, functional moieties and thermal stability by UV-Visible spectrophotometry, X-Ray Diffractometry (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared spectroscopy (FTIR) and Thermo Gravimetric Analysis (TGA) respectively. Cytotoxicity was also determined against LLC PK1 cell lines using LDH assay.

Results: Less aggregate spherical shaped and mono-dispersed nanoparticles were synthesised whose size ranges from 20-30 nm in diameter. Nanoparticles were exhibit face centre cubic crystalline phase with an average size of ~23 nm which was obtained from XRD spectral pattern. Strong interaction between *Desmodium gangeticum* and nanoparticles was shown in TGA-thermogram. The reducing potential and total phenolic content of Ni nanoparticles was found to be same as that of *Desmodium gangeticum*. All the results were expressed as mean±SD of n = 4-6 independent assays, p<0.05, whose data were analysed using ANOVA.

Conclusion: Biological activity of the nanoparticles and its toxicity was assessed and found to possess the good antioxidant and reduction potential with significant antibacterial activity and were nontoxic.

Keywords: Green synthesis, *Desmodium gangeticum*, Ni Nanoparticles, Antimicrobial, DPPH, LLC PK1.

INTRODUCTION

Metal and Metal oxide nanoparticles are now becoming an active area of research especially in the field of regenerative medicine and drug delivery [1]. Ni and its oxide nanoparticles find its significant application in various research areas such as consumer products (batteries and electronics) [2], medical imaging [3], catalysis [4], magnetic storage [5], sensors [6], magnetic ink printing [7] and ferrofluidics [8]. In recent times, Ni nanoparticles have attracted researchers attention due to its high magnetic potential, high magnetoresistance, large coercivities and high Curie temperature.

Ni nanoparticles were synthesized using different approaches such as metal evaporation-condensation [9], electrochemical methods [10], metal salt reduction [11], ultrasound irradiation [12], ultrasound spray pyrolysis [13], polyol methods [14] and neutral organometallic precursor decomposition [15]. Ni nanoparticles can be prepared in various shapes such as nanotubes [16], nano wires [17], nanorods, hollow spheres [18] and nanobelts [19]. In spite of its high yield and better efficiency obtained in conventional synthesis techniques, it has certain limits in terms of toxicity, time consumption, reliability and processing cost. An alternative approach to overcome these limitations is green synthesis involving the exploitation of reducing potential of biomolecules from plants, fungi and bacteria which pave the way for simplicity, economical, less toxicity and feasibility.

The main concern in the synthesis of green nanoparticle is the use of reducing agent like NaBH₄ [20] and N₂H₄, which are mainly involved in the chemical synthesis of nickel nanoparticles. All of these are highly reactive chemicals and pose potential environmental and biological risks. Here, the reducing property of aqueous extract of DG has been exploited for the biosynthesis of nanoparticles.

Desmodium gangeticum (DG) from the Fabaceae family is found mainly in India, China, Africa and Australia. DG has very good medicinal values and thus being used in the Indian system of medicines since ancient times [22]. *Desmodium gangeticum* with

properties like anti-inflammatory [23], anti-analgesic [24] and antioxidant activity [25]. It is found to be very effective against numerous disease conditions viz., jaundice, rheumatism, puerperal fever, paralysis, edema, filaria [26], ischemia reperfusion injury [27] and myocardial infarction [28]. Flavonoids, alkaloids and polyphenol compounds in plants plays a vital role in the preparation of nanoparticles due to its high reducing potential [29]. Pterocarpenoids, gangetin and desmodin, are the major chemical constituents of the *Desmodium gangeticum* root [30].

MATERIALS AND METHODS

NiCl₂, DPPH, Folin-Ciocalteu reagent, Na₂CO₃, K₃ [Fe(CN)₆], TCA (TriChloro Acetic acid) and ethanol were purchased from Sigma Aldrich, India. Muller Hinton agar and agar were purchased from HiMedia, India. LLC PK1 cell line was ordered from NCCS, Pune, India. All the chemicals were used as such without any further purification.

Preparation of aqueous extract

Desmodium gangeticum (Linn) DC. roots were collected from the botanical garden of St. Berchmans College, Changanassery, Kerala, India and authenticated by HOD, Botony department, SB college. The voucher of the specimen was kept for reference. The collected roots were washed thrice thoroughly with tap water and dried in the shade. Roots were cut into small pieces and grinded coarsely using pulveriser. Soxhelt extraction was carried out to obtain crude aqueous extract. The obtained sample is vapourized and lyophilized for the further purpose.

Synthesis of Ni nanoparticles

Ni nanoparticles were synthesized by the simple reduction method. NiCl₂ solution and crude aqueous DG extract was mixed in three different ratio, 1:2, 1:5 and 1:10. The mixture was heated at 80 °C under thorough stirring for around 45 min till the colour of the solution turns from brown to grey. Thus prepared nanoparticles were dried, ground well and obtained the fine powder and were stored for further analysis.

Characterization of Ni nanoparticles

Ni nanoparticles were analysed for its characteristic maximum wavelength (λ) using UV visible spectrophotometer. The absorbance was read out in a wide range of wavelength from 200 nm and 800 nm for the synthesized nanoparticles. FTIR analysis was done to determine the chemical entities and functional groups involved during the synthesis procedure. The spectrum was recorded (Perkin Elmer spectrum 1) between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} . Crystallinity nature of the synthesized nanoparticles was studied using X-ray diffractometry (XRD (Ultima IV, Rigaku). Average size and phase purity were determined. XRD pattern was recorded in a wide range of Bragg's angles 2θ from 10° to 80° at a scanning rate of 2°min^{-1} . The size and morphology of Ni nanoparticles were studied using scanning electron microscope (SEM) (JEOL-JSM 7601F, Japan). The micrographs of nanoparticles were analysed with "ImageJ 1.34s" software. Preparation of stable nanoparticles has become the significant demand in the current research interest. To determine the thermal stability of the nanoparticles and to study about the behaviour of the stabilizing molecules, thermogravimetric (TGA) analysis was performed using SDT-Q600 model, TA instrument USA. Analysis was carried out under nitrogen atmosphere at a heating rate of $10^\circ \text{C}/\text{min}$ and thermogram was recorded between 40°C and 1000°C .

Free radical scavenging, total phenol and reducing power assay

The free radical scavenging effect of DG extract and nickel nanoparticles on DPPH free radical was carried out according to Solver-Rivas *et al.* (2000) with slight modification [31]. Total phenol was measured by the Folin-Ciocalteu reagent in alkaline medium [32]. Reducing potential of biosynthesized nickel nanoparticles and DG extract was determined by Oyaizu method [33].

Antimicrobial activity

Antibacterial activity of the Ni nanoparticles was determined using agar well diffusion method [34]. 25 mL of molten and cooled Muller-Hinton agar were poured in sterilized petri plates. The plates were left overnight at room temperature to check for any contamination. All the bacterial test organisms (*Bacillus subtilis* MTCC-2413, *Escherichia coli* MTCC-574 and *Staphylococcus aureus* E1 Env. Isolate 3) were grown in nutrient broth for 24 hr. A 100 μl nutrient broth cultures of each bacterial organism were used to prepare bacterial lawns in petri plates. Agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 100 μl of Ni nanoparticles, precursor (NiCl_2) and aqueous DG extract. Ciprofloxacin (1 mg/ml) was used as control. The inoculated plates were incubated at 25°C and checked for the zone of inhibition.

Toxicology

Cytotoxicity of Ni nanoparticle was determined by Lactate dehydrogenase activity (LDH) in the culture medium. This assay determines the release of cytoplasmic enzyme lactate dehydrogenase from the plasma membrane to the culture media due to its leakage from the damaged cells due to the presence of the nanoparticles. Cells were seeded in 96 well plates and were treated with synthesised Ni nanoparticle, along with precursor (NiCl_2). LLC PK1 cell lines were used. After 48 hours of treatment, small aliquot of the medium was collected and incubated with reaction mixture containing NAD^+ , lactate and phosphate buffer, each 50 μl . The LDH catalyzed conversion results in the reduction of the tetrazolium salt to Formosan (NAD^+ to NADH) which was measured at an absorbance of 340 nm.

The LDH activity was calculated by the formula

$$\text{LDH activity (IU/L)} = \frac{A_T - A_{0.5 \text{ min}}}{T - 0.5} \times 643 \quad \text{---Eqn 3}$$

Where A_T is the absorbance at time "T"

Statistics

Data of DPPH, reducing power, total phenol and LDH assay signify the mean \pm SD of three samples and are representatives of three independent experiments. The data were subjected to an analysis of variance (ANOVA) by Graphpad prism 5.

RESULTS AND DISCUSSION

We prepared less aggregate and stable Ni nanoparticles biogenetically by using the aqueous extract of DG root with spherical shape and with an average size around 23 nm and were stable. The Ni nanoparticles exhibited antimicrobial effect and has high reducing potential and free radical scavenging ability.

Characterization of Ni nanoparticles UV

Synthesis of Ni nanoparticle with DG extract was physically verified by the colour change from green to dark brown. The absorption behaviour of Ni nanoparticles was studied using UV-Vis spectrophotometer. From the observed spectral shift in the absorption band nanoparticles formation was confirmed. Fig. 1A shows the spectral band of Ni nanoparticles with maximum lambda at 270 nm. This is due to characteristic surface plasmon resonance effect. On decreasing the concentration of DG extracts, the maximum absorption band spectrum shift from 380 nm to 270 nm that may be due to the less aggregation of the particles size confinement. Shape of the nanoparticles was found to be spherical and was monodispersed.

XRD

X-ray diffraction analysis was used to identify the phase and crystallinity of nanoparticles. The XRD pattern of Ni nanoparticles is shown in fig. 1B. A sharp diffraction peak emerges at 2θ angles of 44.5° , 51.8° and 76.4° corresponds to [111], [200] and [220] crystal planes respectively which are in good agreement with face centred cubical structure of Ni nanoparticles [21]. The pattern is in accordance with JCPDS file (card number 04-0835). The peaks at 20.41° and 33.47° correspond to the reflections from [001] and [100] crystal planes exhibits cubical structure of $\text{Ni}(\text{OH})_2$ particles [35]. The peak at 36.71° corresponds to [311] plane corresponds to NiO nanoparticle (JCPDS file card number 04-0850) [36]. The particle size estimated based on the Debye-Scherrer equation was 0.5 nm.

FTIR

The interaction between different species and changes in chemical composition of the mixture was investigated by FTIR. Fig. 1C and 1D shows the IR spectra of Ni nanoparticles and DG extract recorded in the range of 400 – 4000 cm^{-1} . The IR spectrum of Ni nanoparticles covers an absorption band at 3222.90 cm^{-1} , 2075.26 cm^{-1} , 1633.70 cm^{-1} , 1403.88 cm^{-1} , 1104.96 cm^{-1} and 673.07 cm^{-1} (fig. 1C) and the DG extract at 3746.92 cm^{-1} , 3404.83 cm^{-1} , 2928.63 cm^{-1} , 2114.53 cm^{-1} , 1622.95 cm^{-1} , 1400 cm^{-1} , 1079.11 cm^{-1} , 1027.21 cm^{-1} and 671.49 cm^{-1} (fig. 1D). It is found that the absorption peaks of Ni nanoparticles at 3222.90 cm^{-1} , 1633.70 cm^{-1} , 1403.88 cm^{-1} and 673.07 cm^{-1} corresponds to the absorption peaks that are obtained for DG extract. Strong absorption peak at 3222.90 cm^{-1} , 1633.70 cm^{-1} , 1403.88 cm^{-1} , 1104.96 cm^{-1} and 673.07 cm^{-1} refers to O-H stretching, cross linked C-C stretching, stretching vibration of C=O group, C-O stretching and C-Cl stretching, respectively [37]. The above result implies that there is interaction between nanoparticles and aqueous DG extract and further evaluated by TGA.

Thermo gravimetric analysis

The existence of physicochemical moisture either in the form of physical adsorption or in the chemically bound hydroxyl group was analysed by thermo gravimetric technique and the thermo-gram is shown in fig. 2. DG extract showed a linear weight loss till 700°C (fig. 2B). However, bound onto the Ni nanoparticle surface, DG extract exhibits a delayed decomposition. At 700°C , DG extract showed 65% weight loss while Ni nanoparticles showed 40% weight loss. Three significant decays are observed which are of about 11.8%, 16.82% and 30.64% in the range $90-180^\circ \text{C}$, $310-650^\circ \text{C}$ and $680-780^\circ \text{C}$. The weight loss around 100°C corresponds to the evaporation of water molecules and organic compounds. A weight loss at 160°C is observed due to the evaporation of triterpene- β amyron, a phytoconstituent reported in DG extract [38]. The decay at 170°C corresponds to Stigmasterol, a major sterol constituent in DG extract [39]. Another decay of the component was found at the range between 297 to 298°C which may be the decay of isoflavone, Genistein [30]. A weight loss observed at 316°C corresponds to

quercetin, 7-O- β -D-glucopyranoside of DG extract. Further IR spectrum corresponds to Ni nanoparticles (fig 1C). The results thus

indicate that there is a strong interaction between DG extract and Ni nanoparticles.

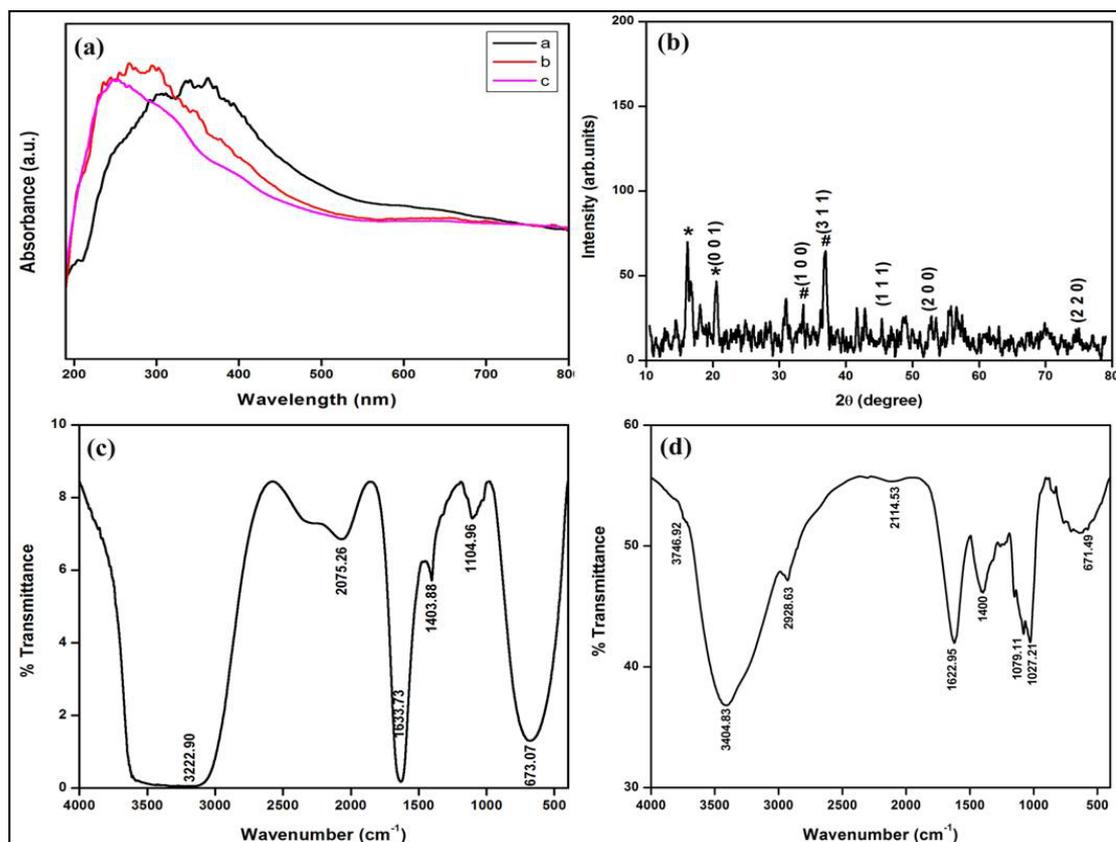


Fig. 1: Characterization of Ni nanoparticles by UV-Visible spectrophotometry, X-Ray Diffractometry and Fourier Transform Infrared Spectroscopy (1A) UV-Visible spectrum of three concentrations of Ni nanoparticles was measured at a range of 300-800 nm. The maximum lambda at 270 nm was observed for 'c' which is in 1:2 ratio concentration; (1B) X-Ray Diffractometry of Ni nanoparticles were measured and showed different peaks at 2θ angles of 44.5° , 51.8° , 76.4° , 20.41° , 33.47° and 36.71° corresponding to the hkl values from 111, 200, 220, 001, 100 and 311 (Rigako instrument); (1C) Fourier Transform Infrared Spectroscopy of Ni nanoparticles were measured between 400 - 4000 cm^{-1} showed peaks at 3222.90 cm^{-1} , 2075.26 cm^{-1} , 1633.70 cm^{-1} , 1403.88 cm^{-1} , 1104.96 cm^{-1} and 673.07 cm^{-1} (Perkin Elmer spectrum1); (1D) Fourier Transform Infrared Spectroscopy of *Desmodium gangeticum* root extracts were measured between 400 - 4000 cm^{-1} showed peaks at 3746.92 cm^{-1} , 3404.83 cm^{-1} , 2928.63 cm^{-1} , 2114.53 cm^{-1} , 1622.95 cm^{-1} , 1400 cm^{-1} , 1079.11 cm^{-1} , 1027.21 cm^{-1} and 671.49 cm^{-1} (Perkin Elmer spectrum1)

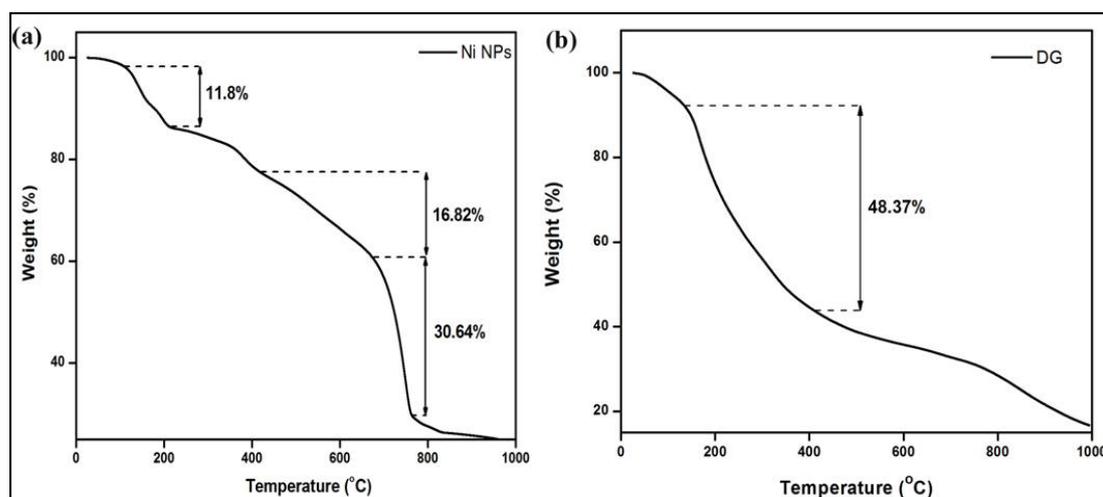


Fig. 2: Thermo Gravimetric Analysis of Ni nanoparticle and *Desmodium gangeticum* root extracts (SDT-Q600 model, TA instrument USA) (2A) Thermo Gravimetric Analysis of Ni nanoparticle showed multiple stage decomposition of about 11.8%, 16.82% and 30.64%; (2B) Thermo Gravimetric Analysis of DG extract showed 80% of its biomolecules decomposed between 80°C - 600°C

SEM

Scanning electron microscopy was used to characterize the mean particle size and morphology of Ni nanoparticle. The analysis of Ni nanoparticle showed less aggregated spherical shaped nanoparticles with a size range from 20 to 30 nm in diameter with a range of 22 nm to 25 nm (fig. 3). Scanning Electron Micrograph (JEOL-JSM 7601F, Japan) shows even distribution of nanoparticles with an average size of around 23 nm and also spherical morphology of the synthesized Ni nanoparticles could be observed with less aggregation.

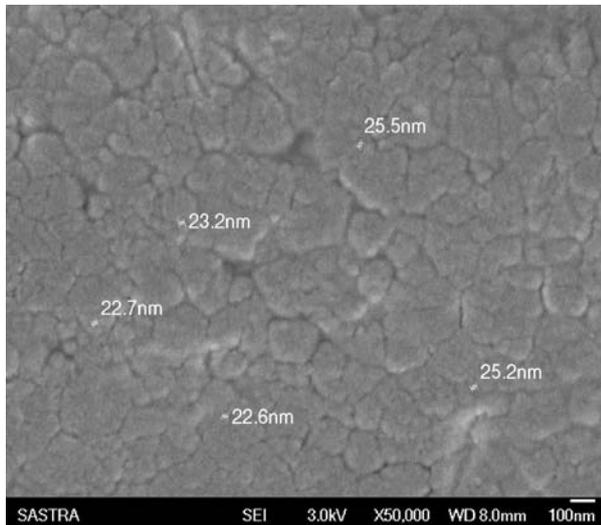


Fig. 3: Scanning Electron Micrograph of Ni nanoparticles

DPPH, Total phenol determination and Reducing potential

Free radical scavenging activity is determined by the capability to scavenge free radical. From fig. 4, gradual decrease in the scavenging activity with the decline in concentration of Ni nanoparticle can be seen. The antioxidant activity of Ni nanoparticles was significantly high as compared its precursor (NiCl₂) but lower than the aqueous DG extract.

Total phenolic content was determined by the spectrophotometric method using Folin-Ciocalteu reagent and the results are shown in fig. 5A. The phenolic concentration of Ni nanoparticles was found to be 2.031 mg/ml and for aqueous DG root extract it was 1.945 mg/ml. In fact both Ni nanoparticle and DG extract showed relatively same reduction potential (fig. 5B) and predict, high antioxidant potential which was reflected in DPPH results (fig. 4). The DPPH activity was calculated by.

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

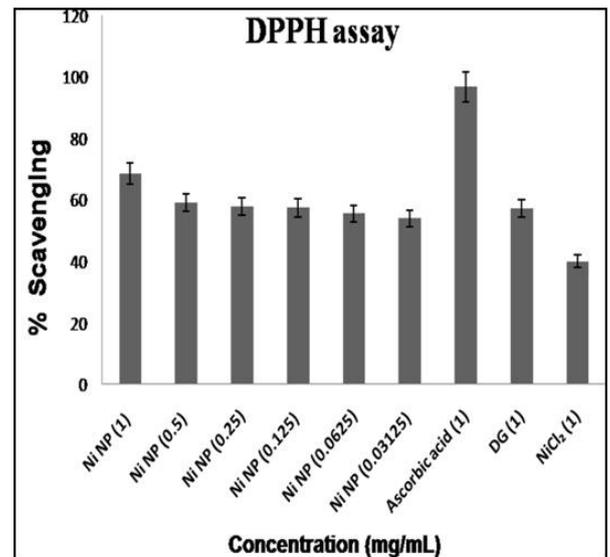


Fig. 4: DPPH Scavenging Activity

DPPH scavenging activity of Ni nanoparticles at various concentrations (1 to 0.03125 mg/ml) and the precursors (NiCl₂ and *Desmodium gangeticum* root extract) for 1 mg/ml concentration were expressed in percentage activity as shown in the graph.

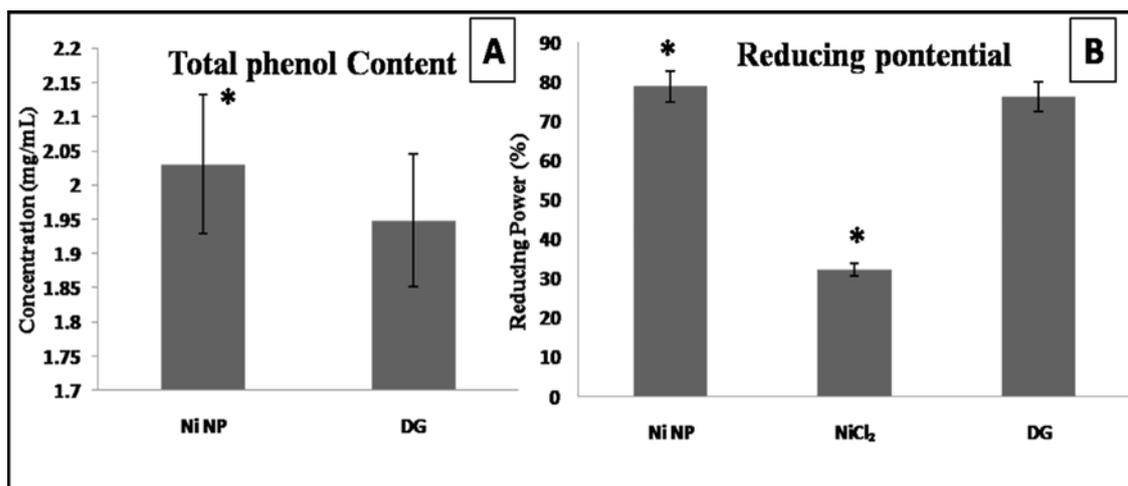


Fig. 5: Total Phenol determination and Reducing potential

(5A) Total phenolic content for Ni nanoparticle (NP) and *Desmodium gangeticum* (DG) root extract was expressed in the terms of concentration (mg/ml). The results were expressed as mean±SD of n = 4-6 independent assays, p<0.05, statistically different from the

Desmodium gangeticum root extract; (5B) Reducing potential of precursors NiCl₂ and *Desmodium gangeticum* (DG) root extract and the Ni nanoparticle were shown in graph in terms of percentage activity of reducing power.

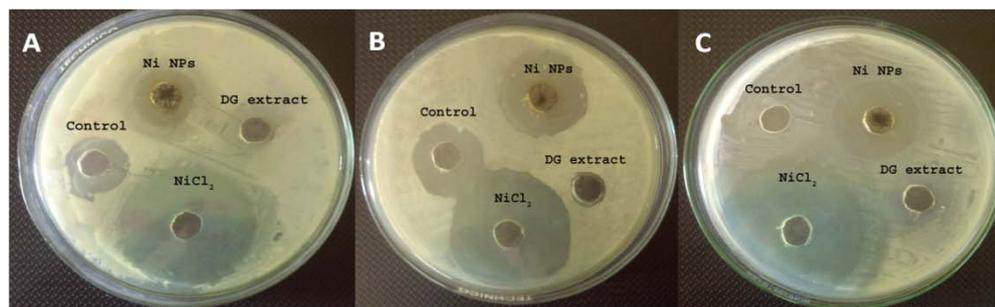


Fig. 6: Antimicrobial assay – Well diffusion method

Antimicrobial activity

Agar well diffusion method was used to determine the antimicrobial activity of Ni nanoparticle against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus* E1) and Gram negative (*Escherichia coli*) bacteria. The zone of inhibition was measured in the incubated petri plates using the software J (National Institutes of Health) and the values are tabulated and shown in the fig. 6. It is seen that the Ni nanoparticles have more effect against *Staphylococcus aureus* E1, followed by *Escherichia coli*. However, compared to the precursor NiCl_2 , Ni nanoparticles showed less

antimicrobial activity. On the other hand, the nanoparticle showed notable activity when compared to the aqueous DG extracts and the control. This may be due to the smaller size and high surface to volume ratio of the Ni nanoparticles which gets incorporated into the cell membrane and cause damage to the bacteria and eventually leads to death. The above fig. illustrates zone of inhibition of three different strains for Ni nanoparticles, precursors (NiCl_2 and *Desmodium gangeticum* (DG) root extract) and the control ciprofloxacin. **6A)** *Bacillus subtilis*; **6B)** *Escherichia coli*; **6C)** *Staphylococcus aureus* E1. The zones were measured using "ImageJ 1.34s" software.

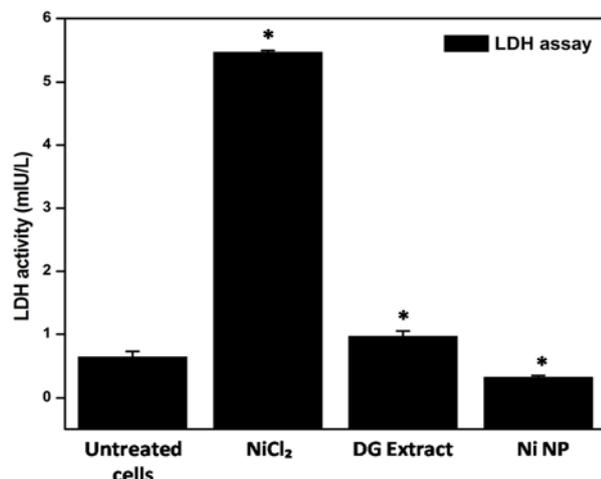
Table 1: Antimicrobial activity (Zone of inhibition)

Strains	Ni NP (in mm)	Control (in mm)	DG extract (in mm)	NiCl_2 (in mm)
<i>Bacillus subtilis</i> (MTCC-2413)	17.831	16.638	11.488	33.282
<i>Escherichia coli</i> (MTCC-574)	23.121	18.343	10.407	36.901
<i>Staphylococcus aureus</i> E1	25.254	17.669	10.038	22.954

The table depicted the zone of measurement of antimicrobial activity for strains *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* E1 for Ni nanoparticles, precursors (NiCl_2 and *Desmodium gangeticum* (DG) root extract) and the control ciprofloxacin.

Toxicology

Fig. 7 shows the activity of released lactate dehydrogenase in cell culture medium upon incubation of LLC PK1 with Ni nanoparticle. The results suggests that Ni nanoparticle has less toxic effect than its precursors DG extract or NiCl_2 . The interaction between phytomolecules and Ni ions reduces the toxicity caused by precursor, NiCl_2 . Thus the synthesized nanoparticles are found to be safe for further medical applications.



*The values are represented as mean \pm SD, n=3

Fig.7: Cellular damage-LDH activity

Fig. 7: shows LDH leakage in the cell culture medium after treated with Ni nanoparticles and its precursors, NiCl_2 and *Desmodium gangeticum* root extract. Ni nanoparticle had less activity on cell lines than the precursors. The result was expressed as mean \pm SD of n = 4-6 independent assays, p<0.05, statistically different from the normal control group (no treatments were made).

CONCLUSION

In summary, we provided a novel cost effective green approach for the synthesis of Ni nanoparticle using *Desmodium gangeticum* aqueous root extract. We prepared stable nanoparticle aggregates with spherical geometry and size of around 23 nm. The synthesised nanoparticle is safe for the use as it did not show any significant cytotoxicity in LLC PK1 epithelial cells. Further the presence of phenolic content and the resultant reducing property not only render antioxidant property but also makes it good antimicrobial agent.

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CONFLICT OF INTERESTS

Declared None

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