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Synthesis of Nanoparticles from wild weeds and assessing its antimicrobial Activity

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Abstract : *The development of the biological experimental process for the synthesis of nanoparticles has evolved a new realm and has expanded into a new and important branch of nanotechnology. The present study aims to analyse the Biological synthesis of nanoparticles using Trifolium and Albizia plant extract and the effect of plant extract in reduction mechanism and particle's crystal structure. The bio-reduction behaviour of plant extracts of Trifolium and Albizia in the synthesis of Cu, Fe and Ag was investigated employing UV/visible spectrophotometry, XRD and Fourier transform infrared spectroscopy.*

Keywords: *Nanoparticles, Phytochemicals, Antimicrobial, UV/Vis , XRD , FTIR.*

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Introduction:

Nanotechnology is the science that deals with matter at the scale of one billionth of a metre i.e 10^{-9} m equals to 1nm and is also the study of manipulating matter at the atomic and molecular scale .

A nanoparticle is the most fundamental component in the fabrication of a nano-structure and is far smaller than the world of everyday objects that are described by Newton's laws of motion but bigger than an atom or a simple molecule that are governed by quantum mechanics.

Nanoparticles are fundamental building blocks of nanotechnology.The most important and distinct property of nanoparticles is their larger surface area to volume ratio(Arangasamy leela et al 2008).

Physical and chemical methods for synthesis of nanoparticles are more popular but they lead to the synthesis of toxic compounds which limits their application and it is not also economically feasible.

Biosynthesis of nanoparticles is now established as an alternative to chemical and physical methods of synthesis (Peter Amaladhas et al 2012).

Metallic nanoparticles have different physical and chemical properties from bulk metals, eg:lower melting point,higher specific surface areas,specific optical properties,mechanical strengths and specific magnetizations.

These properties would result in interesting new applications of metal nanoparticles that could potentially be utilized in the biomedical sciences and areas such as optics and electronics.

Nanoparticles of free metals have been extensively researched because of their unique physical properties, chemical applications in catalysis, biological labelling, bio-sensing, drug delivery, anti-bacterial activity, anti-viral activity, detection of genetic disorder, gene therapy and DNA Sequencing (Singh et al 2013).

Methodology :

Plant material: The leaves of two different weed plants namely *Albizia lebbeck* and *Trifolium repens* were collected from the campus of PATNA WOMEN'S COLLEGE. The leaves were washed there in the tap water and then with double distilled water to remove the external dirt and kept for drying in hot air oven at 60°C for 5 days. After that the dried leaves were ground to powdered form and stored in air tight containers at room temperature (Prasad et al 2010).

Phytochemical Screening : Qualitative phytochemical analysis was done by using the procedures of Kotate et al (1995). The following tests were performed on extracts to detect various phytochemicals present in them.

1. DETECTION OF FLAVONOIDS:

Ferric Chloride Test:Test solution when treated with a few drops of Ferric Chloride solution would result in the formation of blackish red colour indicating the presence of flavonoids.

2. DETECTION OF ALKALOIDS:

The extract was mixed with ammonia and then with chloroform solution. To this dilute HCL was added. The acid layer was used for chemical test for alkaloids.

Mayer's Test: The acid layer with a few drops of Mayer's reagent gives a creamy white ppt.

3. DETECTION OF TANNINS:

To 5 ml of extract, $FeCl_3$ was added. Formation of deep blue or greenish black colour shows the presence of tannins.

4. DETECTION OF GLYCOSIDES:

Kellar Killani's Test: Dissolved the extract in water with glacial acetic acid and $FeCl_3$ and conc. H_2SO_4. They give a brown ring at the junction.

5. DETECTION OF STEROIDS :

Salkowski's of Steroids: To 2 ml of the extract, 2 ml of chloroform was added followed by 3 ml of H_2SO_4. A reddish brown colour indicated the presence of steroids.

6. DETECTION OF PHENOLS:

Test extract (50mg) was dissolved in 5 ml of distilled water. To this a few drops of neutral 5% $FeCl_3$ solution was added. A dark green colour indicates the presence of phenolic compound.

7. DETECTION OF SAPONINS:

A pinch of dried powdered plant was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponin.

8. DETECTION OF CARBOHYDRATES:

Molish Test: To the powdered plant extract 1 ml of -Napthol solution conc. H_2SO_4 was added to the sides of test tube. Purple or reddish violet colour forms at the junction.

9. DETECTION OF PROTEINS:

Xanthoprotein Test: To a few mg of powder, 1 ml conc. HNO_3 was added. It was then boiled and cooled. Formation of white ppt. Indicates the presence of protein.

10. DETECTION OF AMINO ACID:

3 ml of test solution and 3 drops of 5% Ninhydrin solution were added in a test tube and heated in water bath for 10 minutes. Formation of purple or bluish colour indicates the presence of amini acid.

PREPARATION OF PLANT EXTRACTS: 5 gm each of powdered plant materials were added to 100 ml distilled water and then filtered to get extract .

SYNTHESIS OF SILVER, COPPER AND IRON NANOPARTICLES: 5 gm of powdered sample was dissolved in 100ml of distilled water and boiled at 60° C for 5 minutes. The resulting crude extract was filtered through Whatmaan No. 1 filter paper. 25ml of plant extract was added to 100ml of 1mM $AgNo_3$ (for silver) (Gandhi et al 2014), 1mM $CuSo_4$ (for copper) and 0.01M $FeCl_3$ (for iron)solution was kept in microwave at 90° C for 15 minutes and the colour change was observed.

CHARACTERIZATION OF NANOPARTICLES : The biogenic synthesised nanoparticles were characterized using UV-Vis spectroscopy, XRD and FTIR. UV-Vis spectra analysis was done by using UV-Vis spectrophotometer at the range of 200-800nm (Renugadevi et al 2012).

ANTIMICROBIAL ACTIVITY: The two bacterial strains used in our study are *Staphylococcus aureus* (gram+ve) and *Escherichia coli* (gram-ve). They were obtained from IGIMS, Patna. The effect of Silver , Copper and Iron nanoparticles were arranged by agar diffusion method and further confirmed by disc diffusion method.

Result and Discussion :

Phytochemical Analysis:

The results obtained for phytochemical screening were tabulated below:

Synthesis of nanoparticles:

In the present study , *Albizia lebbeck* and *Trifolium repens* leaf extract were used as reducing agents for the synthesis of silver , copper and iron nanoparticles respectively. Silver nanoparticles were successfully synthesised in ***Albizia*** , copper nanoparticles in *Albizia* and iron nanoparticles in *Trifolium*. Further these synthesised nanoparticles were characterized by UV-Vis spectral analysis at the range of 200-800 nm using UV-Vis Spectrophotometer.

In case of Silver nanoparticles , the colour changed from colourless to dark orange in *Albizia*. The synthesis of Ag nanoparticles was confirmed by measuring the UV-Vis spectrum of the reaction media. As shown in **Fig 1** , the absorption spectrum of the Ag nanoparticles solution showed surface Plasmon band of 400 nm , confirmed the synthesis of Ag nanoparticles in *Albizia*. Similar results were observed (Ahmad et al 2012) in which silver nanoparticles was synthesised using *Punica granatum.*

In case of Copper nanoparticles , colour change from orange to brown colour confirmed the presence of Cu nanoparticles. The UV-Vis spectra of Cu nanoparticles synthesised using *Albizia lebbeck* showed absorption peak maximum at 400 nm in **Fig 1**. Similar results were obtained for the synthesis of Cu nanoparticles on the *Capparis zeylanica* plant with little variation (Saranyaadevi et al 2014).

The leaf extract of *Trifolium repens* was found to be the most suitable for synthesis of Iron nanoparticles. Colour change was observed from green to brownish black indicating the formation of Fe nanoparticles. As shown in **Fig 1**, spectrophotometer of Iron nanoparticles produced a peak centered near 200 nm. More or less similar results were noticed during the synthesis of Fe nanoparticles in previous work using various species (Pattanayak et al 2013).

Analysis through X-Ray Diffraction was carried out to confirm the crystalline nature of the nanoparticles.

As shown in Fig. 2 and 3, the presence of structural peaks in XRD patterns and the average crystalline size clearly illustrate that the nanoparticles synthesised were nanocrystalline in nature.

FTIR: Infrared Spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance. The particles were analysed under FT-IR for the size conformation. It is an effective analytical technique for detecting functional groups and characterizing covalent bonding information.

ANTIMICROBIAL ACTIVITY: Bio-synthesised Silver, Copper and Iron nanoparticles were analysed for antimicrobial activity against *E.coli* and *Staphylococcus aureus* by Disc-Diffusion method.

In our present investigation, we have studied the antimicrobial activity of Silver, Copper and Iron nanoparticles using two different weeds. In a similar study (Balashanmugam et al 2013), which was the synthesis of Silver nanoparticles from orange peel extract and its antimicrobial activity against fruit and vegetable pathogen, a large zone of inhibition was observed against *E.coli*. In the present study also, all the two nanoparticles were most active against *E.coli*, where the highest zone of inhibition was 16 mm in case of Ag nanoparticles, 14 mm in case of Cu and 14 mm in case of Fe nanoparticles.

Significance :

Achievement of such plant mediated synthesis of nanoparticles has made the approach more efficient as it has many advantages, such as, ease with which the process can be scaled up , economic viability etc.

It could be an eco friendly alternative to chemical and physical method which involves a myriad of chemicals.

In the present study it was found that Ag, Cu, Fe, nanoparticles can be synthesised from 1mM $AgNo_3$, 1mM $CuSo_4$ and 0.01 M $FeCl_3$ solution respectively using leaf and stem extract of *Albizia lebbeck* and *Trifolium repens*.

The successfully biogenic synthesised nanoparticles showed antibacterial activity on both Gram positive and Gram negative bacteria. Thus, these particles can be used to treat certain microbial diseases after further analyses .

The phytochemical constituents present in noxious weeds could act as potential source of useful drugs to improve the health status of humans.

Thus, it is concluded that , the wild weed could be a potential source of phyto-constituent for the synthesis of nanoparticles.

LIST OF TABLES

Table. 1. Phytochemical analysis of distilled water extracts of *Albizia lebbeck* and *Trifolium repens*.

Phytochemical	*Albizia*	*Trifolium*
1. Flavonoids	+	-
2. Alkaloids	-	-
3. Tannins	+	-
4. Glycosides	-	-
5. Steroids	-	+
6. Phenolic	+	-
7. Soponins	+	+
8. Carbohydrates	+	+
9. Proteins	+	+
10. Amino acids	-	-

Table 2. Antimicrobial activity of nanoparticles

Name of bacteria	Diameter of zone of inhibition (mm)			
	Streptomycin (control)	Silver nano-particles	Copper nano-particles	Iron nano-particles
		Albizia	*Albizia*	*Trifolium*
Gram-ve*(E.coli)* Gram+ve	21	16	14	14
(Staphylococcus aureus)	19	13	13	11

LIST OF FIGURES

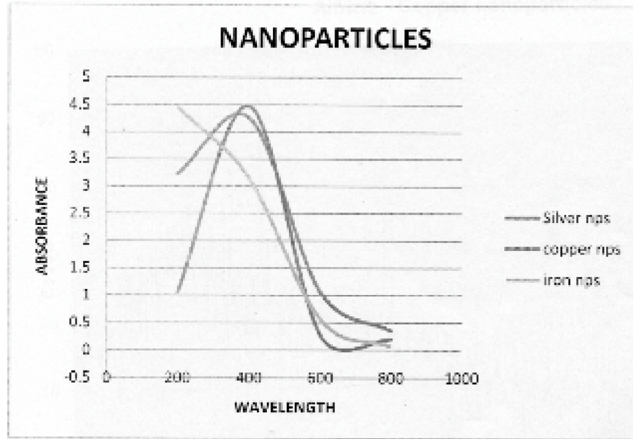

Fig. 1. UV-Vis absorption of spectra of Silver, Copper and Iron nanoparticles by weeds

Albizia lebbeck :

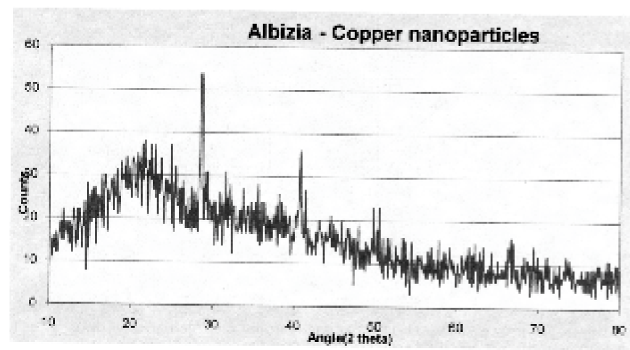

Fig. 2. XRD pattern of Copper nanoparticles by *Albizia*

Trifolium repens:

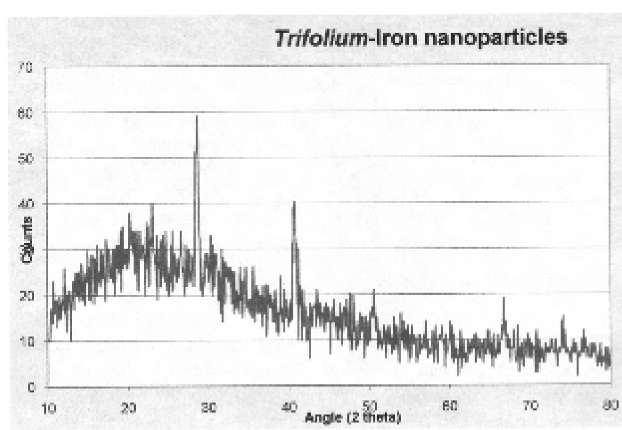

Fig. 3. XRD pattern of Iron nanoparticles by *Trifolium*

***Albizia lebbeck*:**

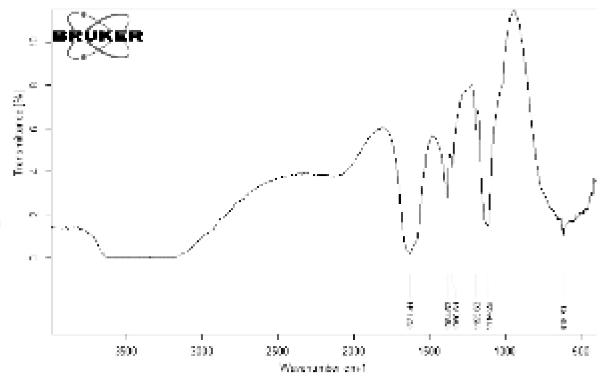

Fig. 4. Graph indicating the conversion of copper sulphate into Copper nanoparticle for *Albizia* leaf

***Albizia lebbeck*:**

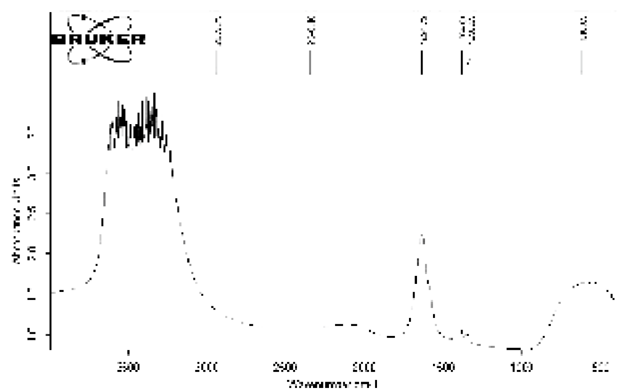

Fig. 5. Graph indicating the conversion of silver nitrate to Silver nanoparticle for *Albizia* leaf

***Trifolium repens*:**

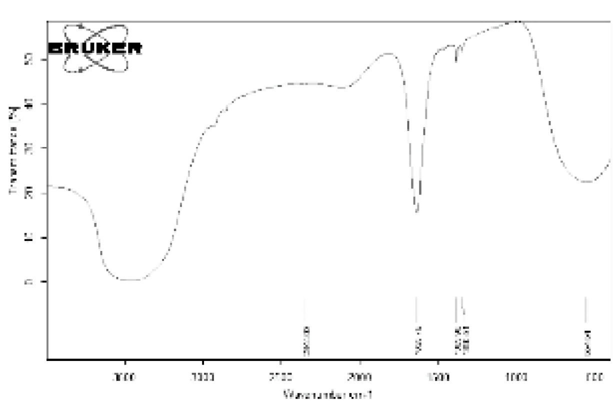

Fig. 6. Graph indicating the conversion of ferric chloride into Iron nanoparticle for *Trifolium* leaf

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