

Comparison of Silver, Copper and Iron Nano particles from Leaf Extract of *Azadirachta indica* and its Anti-microbial, Bio sensing activity and toxicity

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ABSTRACT: Nanotechnology is an emerging field in Biotechnology and it has various applications in all other fields as well. There are a number ways of synthesizing nanoparticles; one such way is using biomolecules from plant extracts to reduce metal ions to nanoparticles in a single step. The chemical reaction usually involves organic compounds like Flavonoids, Alkaloids, Phenols etc., reacting with metal ions to create a metal nano-particle. The chemical constituents of plant extracts in the process act as reducing agents as well as stabilizing agent for the nano-particle. The process is quick, easy and can readily be scaled up. In this study, the leaves of *Azadirachta indica* were used for the synthesis of silver, copper and iron nanoparticles. A qualitative analysis of the plant extract was done to know about its bioactive compounds present in it. Synthesis of nano particles was done using prior precursors like silver nitrate, copper sulphate and ferric chloride. The synthesized nanoparticles were characterized by UV-Visible spectrophotometer at the range of 200-800nm. The absorbance peak was measured. The absorbance peak of silver nanoparticle was measured at 434nm followed by copper and iron at 544nm and 282nm. Antimicrobial activity was done against 3 gram-positive and 3 gram-negative bacteria and 2 fungal species. Silver nanoparticles showed inhibition against all the microbial culture with maximum inhibition of 25mm against bacteria *Enterobacter faecium*. The iron nano particle showed minimum inhibition to only four microbial cultures. Haemolytic activity was done to detect the toxicity of the synthesized nanoparticle and it clearly shows that the nano particles of concentration between 1- 25µg/ml were nontoxic and the toxicity increases with the concentration above 25µg/ml. The genotoxicity study shows that all the concentration of all samples except iron (5%) were not genotoxic. The biosensing activity shows that these nanoparticles are a good source for the biosensing of toxic heavy salts.

KEYWORDS: Nano particles, *Azadirachta indica*, phytochemicals, silver nanoparticles, copper nanoparticles, iron nanoparticles, UV-Vis spectroscopy, Anti-microbial, Toxicity, Biosensing activity

I. INTRODUCTION

Nanotechnology is the term given to those areas of science and engineering where phenomena that take place at dimensions in the nanometre scale are utilized in the design, characterization, production and application of materials, structures, devices and systems. Nano technology is one of the most rapidly progressing fields of technology and it has opened up numerous new frontiers of research for us. Its advent into the field targeted drug delivery, therapeutic actions and as bio sensors has captured the imagination of the scientific community and various methods are being devised to from new nanoparticles with more specifications, scientists are striving to come up with methods which let us control the shape, size, specificity and other characteristics of the particles more closely.

One of the most useful and revolutionary technique coming up presently is synthesis of nanoparticles using plant extracts and their subsequent action. The formation of nano particles using plant extracts has a major edge over methods in terms of its interaction and effect on the environment; it is completely environmentally friendly and does

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not pose any threats even from its waste. The time required for the formation of particles is also within acceptable limits and with the ease of getting the requisite plants make it one of the best options available in this field to develop the particles.

Azadirachta indica, also known as Neem is an evergreen tree of India, which belongs to the plant family Meliaceae. It has been recognized as one of the most promising sources of compounds with insect control, antimicrobial and medicinal properties. In India, Neem has been in use since ancient times as a traditional medicine against various human ailments and about 700 herbal preparations based on neem are found in Ayurveda, Siddha, Unani and other local health prescriptions. Neem has also received a lot of attention worldwide for its potential use as an herbal pesticide and other healthcare formulations in countries such as China, USA, France, Germany, Italy etc.

II. MATERIALS AND METHODS

Collection and Identification of Plant sample

The *Azadirachta indica* leaf (figure: 1) sample was collected from the campus of Dr. N.G.P. Arts and Science College, Coimbatore and it was identified and authenticated by Botanical Survey of India, Coimbatore. (Plant Authentication NO: BSI/SRC/5/23/2018/Tech/2299).



Figure: 1 *Azadirachta indica*

Preparation of Extract for Phytochemical analysis

The leaves of *A. indica* were washed thoroughly in double distilled water to remove the dust particles and were shade dried. The dried leaves were then finely ground into powder. About 10g of powder was weighed and dissolved in water and alcoholic extract like ethanol for phytochemical analysis and kept in orbital shaker at 120rpm for 24 hours. The extracts were then filtered using filter paper and stored at 4°C until use.

QUALITATIVE ANALYSIS OF PRIMARY METABOLITES

Test for Carbohydrates: 1. Benedict's test: About 0.5ml of the filtrate was taken to which 0.5ml of Benedict's reagent was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

2. Molisch's test: To about 2ml of the sample, 2 drops of alcoholic solution of α -naphthol was added and to the mixture after being shaken well. Few drops of conc. H_2SO_4 were added along the sides of the test tube. A violet ring indicates the presence of sugars.

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Test for Proteins

1. Biuret test: 2ml of filtrate was taken to which 1 drop of 2% copper sulphate solution was added. 1ml of 95% ethanol was added. Then it was followed by excess addition of KOH. The appearance of pink colour indicates the presence of proteins. 2. 2ml of extract was mixed with 2ml of water and about 0.5% of conc. HNO_3 was added. The appearance of yellow colour indicates the presence of proteins.

Test for amino acids

1. To 1ml of the extract, few drops of ninhydrin reagent (10mg of ninhydrin in 200ml of acetone) was added. The appearance of purple colour indicates the presence of amino acids.

Miscellaneous compounds

Test for resins: 1ml of extract was taken and to this few ml of acetic anhydride was added. To this 1ml of conc. H_2SO_4 was added. The appearance of orange to yellow colour indicates the presence of resins.

Test for fixed oils and fats

1. Spot test: Small quantity of the extract was taken and pressed between 2 filter papers. The appearance of spots indicates presence of oils.
2. Saponification test: To the extract, few drops of 0.5N alcoholic KOH and few drops of phenolphthalein was added. This mixture was heated for about 2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils or fats.

Test for Gums and Mucilage: To 1ml of extract, distilled water, 2ml of absolute ethanol was added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

Test for Carboxylic acids: To 1ml of extract a pinch of sodium bicarbonate was added. The production of effervescence indicates the presence of carboxylic acids.

QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

Test for Alkaloids

1. Mayer's test: To a few ml of filtrate, 2 drops of Mayer's reagent was added. A creamy white precipitate shows a positive result for alkaloids. 2. Wagner's test (iodine-potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish-brown precipitate indicates presence of alkaloids. 3. To 5ml of extract, 2ml of HCL was added. Then, 1ml of Dragendroff's reagent was added. An orange or red precipitate shows a positive result for alkaloids.

Test for Glycosides

1. Borntrager's test: To 2ml of filtrate, 3ml of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added. The pink colour indicates the presence of glycosides. 2. 5ml of extract was hydrolysed with 5ml of conc. HCL boiled for few hours in a boiling water bath. Small amount of alcoholic extract was dissolved in 2ml of water and aqueous 10% NaOH was added. The presence of yellow colour was a positive result for the glycosides. 3. 2ml of extract is mixed with about 0.4ml of glacial acetic acid containing traces of H_2SO_4 was added. The production of blue colour is positive for glycosides.

Test for Cardiac glycosides (Keller-Killani test)

5ml of solvent extract was mixed with 2ml of glacial acetic acid and a drop of ferric chloride solution was added followed by the addition of 1ml of conc. H_2SO_4 . A brown ring in the interface indicates the presence of deoxy sugars of cardenolides. A violet ring may appear beneath the brown ring while acetic acid layer a green ring may also form just gradually towards the layer.

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Test for Phenol

1. Gelatine test: To 5ml of extract, 2ml of 1% solution of gelatine containing 10% of NaCl is added. Appearance of white precipitate indicates the presence of phenol.
2. Lead acetate test: To 5ml of extract, 3ml of 10% lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for phenols.

Test for Polyphenols:

1. To the 3ml of the extract 10ml of ethanol was added and were warmed in a water bath for 15 minutes. To this few drops of ferric cyanide (freshly prepared) was added. The formation of blue – green colour indicates the presence of polyphenols.
2. To 1ml of extract few drops of 5% solution of lead acetate was added. The appearance of yellow precipitate indicates the positive result for polyphenols.
3. To the 5ml of ethanolic extract, 0.1% gelatine solution was added. The formation of precipitate was positive for polyphenols.

Test for tannins

To 5ml of extract, few drops of neutral 5% ferric chloride solution were added. The production of dark green colour indicates the presence of tannins.

Test for flavonoids

1. To the aqueous solution of the extract, 10% of ammonia solution was added and heated. The production of fluorescence yellow is positive of flavonoids.
2. 1ml of extract was taken and 10% of lead acetate was added. The yellow precipitate is positive for inference for the flavonoids.
3. The extract is treated with conc.H₂SO₄ resulting in the formation of orange colour indicates the positive result for flavonoids.
4. To 5ml of dilute ammonia, the plant extract is added and shaken well. The aqueous portion is separated and conc.H₂SO₄ was added. The yellow colour indicates the presence of flavonoids.

Test for Phytosterols

1. The extract is dissolved in 2ml of acetic anhydride and to which 1 or 2 drops of conc.H₂SO₄ was added along the sides. An array of colour change indicates the presence of Phytosterols.
2. The extract was refluxed with alcoholic KOH and saponification takes place. The solution was diluted with ether and the layer was evaporated and the residue was tested for Phytosterols. It was dissolved in dilute acetic acid and few drops of conc.H₂SO₄ are added. The presence of bluish green colour indicates the presence of polysterols.

Test for Phlobatannins

Aqueous extract was boiled with diluted HCL leading to the deposition of reddish precipitate indicates the presence of Phlobatannins.

Test for saponins

1. 0.5mg of extract was vigorously shaken with few ml of distilled water. The formation of frothing is positive for saponins.
2. The froth from the above reaction is taken and few drops of olive oil was added and shaken vigorously and observed for the formation of emulsion.

Test for Steroids

To 2ml of the extract, 2ml of chloroform and 2ml of conc.H₂SO₄ was added. The appearance of red colour and yellowish green fluorescence indicates the presence of steroids.

Test for xanthoproteins

1ml of extract is taken and to this few drops of nitric acid and ammonia are added. Reddish brown precipitate indicates the presence of xanthoproteins.

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Test for Terpenoids (Salkowski test)

3ml of extract was taken and 1ml of chloroform and 1.5ml of conc. H₂SO₄ was added along the sides of the tube. The reddish brown colour in the interface is considered as positive for presence of Terpenoids.

Test for Coumarins

To 2ml of the extract, 3ml of 10 aqueous solution of NaOH was added. The production of yellow colour indicates the presence of coumarins.

Green synthesis of Silver nanoparticles

About 50ml of working standard of silver nitrate (0.01M) was taken in a beaker and placed in a magnetic stirrer at 45-50°C for 15minutes. About 0.2 ml of plant extract was added drop by drop to the solution. The production of brown-yellow colour indicates the synthesis of nanoparticles. It was kept in dark at room temperature to prevent agglomeration of the nanoparticles.

Green synthesis of Copper nanoparticles

About 50ml of working standard of copper sulphate (0.01M) was taken in a beaker and placed in a magnetic stirrer at 45-50°C for 15minutes. About 0.5-1ml of plant extract was added drop by drop to the solution. Appearance of green colour indicates the synthesis of copper nanoparticles. It was kept in dark for about 8-12hours before the analysis

Green synthesis of Iron nanoparticles

About 50ml of working standard solution of ferric chloride (0.01M) was taken in a beaker and kept in an magnetic stirrer at 45-50°C for 15minutes. About 20ml of plant extract was slowly added to the solution. It was again stirred for 15-30minutes where the appearance of blackish green colour indicates the production of iron nanoparticles. It was incubated in dark at room temperature for 12 – 24 hours.

Characterization using UV-Visible spectrophotometer

About 1ml of aliquot samples was taken in a quartz crystal cuvette. They were analysed in Shimadzu UV-Visible-NIR Spectroscopy UV 1800. The optical absorbance of bio-reduction of silver ions in the solution was measured in the range of 200-800nm with the sampling interval of 2.0 followed by iron at same wavelength. The results were recorded and the absorption curve was created.

Antimicrobial activity

Antimicrobial activity of the sample was done by well diffusion technique using 3 gram-positive bacteria, 3 gram-negative bacteria and 2 fungal pathogens. The gram-positive bacteria include *Enterobacter faecium*, *Streptococcus pyogenes*, *Staphylococcus aureus* and gram-negative bacteria which includes *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. And two fungal pathogens include *Candida albicans* and *Aspergillus flavus*. The bacterial culture was spread on the Muller-Hinton agar plates and the fungal culture on Sabouraud dextrose agar. The plates were then swabbed with respective pathogens. Wells were cut using well puncture at 6mm in diameter which was then filled with 50µl of synthesized nano particle and also the plant extract in aseptic condition. The plates were then incubated at 37°C for 24 hours for bacterial culture and 28°C for 72 hours for fungal culture. The antimicrobial activity was calculated by measuring the zone of inhibition.

Toxicity Testing

Genotoxicity

The purple coloured varieties of Onion samples were used in this study; the samples were collected and are washed to remove the dirt and were sun dried for 2 weeks and the dried roots present in the tip of the onion were cut to expose fresh meristematic tissues. In different boiling test tubes the synthesized nanoparticles and the extract are taken at various concentrations (1%, 3%, and 5% of the stock solution of synthesized nano particles) and water was added. In the tubes, the onion samples were placed and were allowed to germinate and produce root tips for about 12 days. The freshly produced root tips were taken and about 5 different samples per each bulb is taken and are observed under

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microscope. For staining, the onion root tips were treated with a drop of conc. HCl and were washed with distilled water, then it was gently pressed and stained with safranin and are visualized for any mutation in the nucleus and chromosomes. The results were recorded.

Haemolytic activity

Preparation of erythrocyte suspension

5ml of blood sample was collected from a healthy individual in a tube containing an anti-coagulant. The blood was centrifuged at 1500-2000rpm for 3minutes. The supernatant was discarded and the pellet was washed three times with sterile distilled phosphate buffer saline (pH 7.2 ± 0.2). The cells were resuspended in normal saline to 0.5%.

Activity

0.5ml of cell suspension and 0.5ml of nanoparticles at various concentrations (100, 500, 1000µg/ml) in PBS was added and incubated for 30 minutes at 37°C. The mixture was centrifuged at 1500 rpm for 10 minutes. Free haemoglobin was in the supernatant and measured in UV-Vis spectroscopy at 540 nm. The PBS and distilled water was used as minimal and maximum control.

$$\% \text{Haemolysis} = \frac{At - An}{Ac - An} \times 100$$

Where,

At = Absorbance of test sample

An = Absorbance of saline control

Ac = Absorbance of water control

Biosensing Activity

Heavy metals bio sensing is done using nano particles, for biosensing of silver, iron and copper nano particles; they are treated with 0.01 M solutions of heavy metal salts. 0.01M solutions of heavy metal salts like Mercuric chloride, ferrous sulphate, ferric chloride, lead acetate and zinc sulphate were prepared. About 0.1ml of the solution and 1ml of nano particle were added to the test tube. They were incubated for 24 hours and results were observed. The colour change or precipitate may be formed which can infer the biosensing activity of the nanoparticles.

III.RESULT AND DISCUSSION

In this study, the phytochemicals present in the alcoholic extracts of *Alpinia officinarum* was analysed using standard screening methods. The results reveal that the plant contains various bioactive constituents like phenols, tannins, alkaloids, are present. The phytochemicals characteristics are summarize in the table – 1.

Table-1
Qualitative phytochemical analysis of leaf extract of *Azadirachta indica*

S. No	Phytochemicals	Leaf extract of <i>Azadirachta indica</i>
1.	Carbohydrates	Present
2.	Proteins	Absent
3.	Amino Acids	Absent
4.	Gums and mucilage	Present
5.	Resins	Present
6.	Fixed oils	Present
7.	Carboxylic acids	Absent

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8.	Quinones	Absent
9.	Alkaloids	Present
10.	Glycosides	Present
11.	Cardiac Glycosides	Present
12.	Phenol	Present
13.	Tannins	Present
14.	Flavonoids	Absent
15.	Phytosterols	Present
16.	Phlobatannins	Absent
17.	Saponins	Absent
18.	Steroids	Absent
19.	Xanthoproteins	Absent
20.	Terpenoids	Absent
21.	Coumarins	Present

Synthesis of nanoparticles:

The nanoparticle synthesis was confirmed by the reduction of the ions and the development of the colour. The reduction of silver ions was confirmed by the colour development of brown-yellow after the addition of silver nitrate. This indicated the presence of silver nanoparticles. The reduction of copper ions was indicated by the development of green colour after addition of copper sulphate. . This indicated the presence of copper nanoparticle. The reduction of ferric ions was identified by the development of greenish - black colour of the solution after the addition of ferric chloride which indicated the presence of iron nanoparticles. The colour development of the solution is shown in figure-2.

UV-Visible Spectroscopy

The UV-Visible spectrophotometer is the preliminary characterization of nano particles. The bio reduction of the ions was monitored by measuring the solution in UV-Vis Spectroscopy. It was monitored at 200 to 800nm for all the synthesized nanoparticles which includes silver, copper and iron nano particles. The absorption of the synthesized silver nanoparticle has absorbance peak at 434nm shown in figure-3 which is similar to the other literature available for silver nanoparticles from leaf extract of *A.indica* which showed peak of 450nm. The copper nano particles synthesized showed absorbance peak at 544nm which is quite similar to the copper nanoparticle synthesized from the leaf extract of *Ocimum sanctum* at the peak of 560nm. The synthesized iron nanoparticles from the leaf extract of *A.indica* showed absorbance peak at 282nm which is very nearby to the iron nanoparticle synthesized from the dried fruit extract of *Ficus carica* which showed peak at 290nm. The absorption spectra of synthesized nanoparticles are shown in figure- 3 and 4.

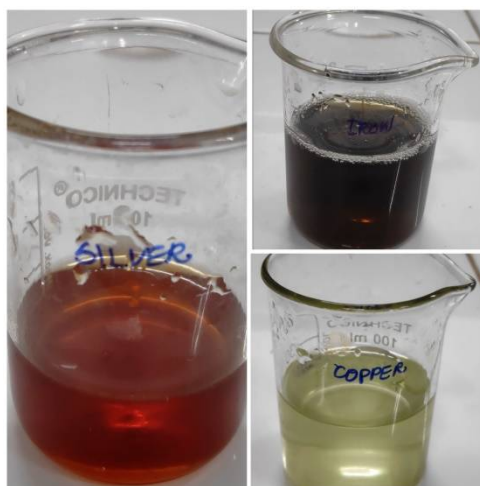


Figure: 2 Synthesis of nanoparticles (Silver, iron and copper)

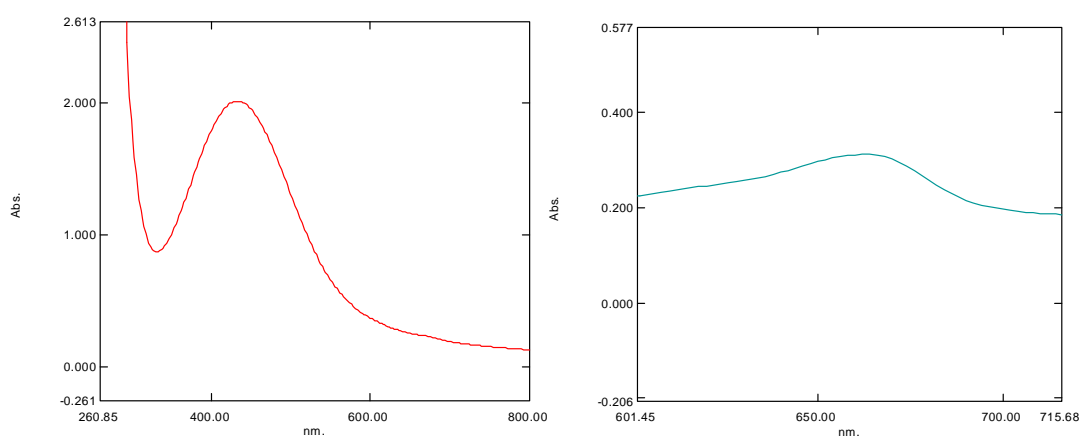


Figure 3, 4: UV-Visible Spectroscopy analysis of silver and copper

Anti-microbial activity

The present study shows the antimicrobial activity against three gram-positive bacteria *Enterobacter faecium*, *Streptococcus pyogenes*, *Staphylococcus aureus*, three gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and two fungal cultures *Candida albicans* and *Aspergillus flavus*. Tetracycline was used as the positive control. The leaf extract of *Azadirachta indica* showed inhibition against two gram-positive bacteria namely *Staphylococcus aureus* and *Enterobacter faecium* and two gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and one fungal culture *Candida albicans*. Maximum inhibition was shown against the bacteria *Enterobacter faecium* with the zone of inhibition of 13mm.

The synthesized silver nanoparticle showed inhibition against all the microbial culture with the maximum inhibition of 25mm against *Enterobacter faecium* bacteria. The copper nanoparticle also showed inhibition against all the culture except *Klebsiella pneumoniae*. The maximum inhibition was shown against the bacteria similar to silver nanoparticle which is *Enterobacter faecium* with the zone of inhibition of 21mm. The iron nanoparticles showed inhibition only against two bacteria *Pseudomonas aeruginosa* and *Enterobacter faecium* with the zone of inhibition of 13mm and 11mm and against two fungal cultures *Candida albicans* and *Aspergillus flavus* with zone of inhibition of 10mm and 13mm. The study reveals that the synthesized silver nanoparticles showed inhibition against all the microbial cultures and the least inhibition was given by the iron nanoparticles. Though silver nano particles showed inhibition against all

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the cultures, the copper nanoparticles showed high zone of inhibition against the fungal cultures than silver nanoparticle. The zone of inhibition against all the cultures is given in the table-2, figure 5, 6.

Table-2
Antimicrobial activity of *Azadirachta indica* and the nanoparticles synthesized from the leaf extract

S. No	Microbial culture	+ Ve Control	plant extract	Silver nanoparticle	Copper nanoparticle	Iron nanoparticle
1.	<i>Enterobacter faecium</i>	30 mm	13mm	25mm	21mm	11mm
2.	<i>Streptococcus pyogenes</i>	25 mm	-	15mm	20mm	-
3.	<i>Staphylococcus aureus</i>	23 mm	10mm	17mm	16mm	-
4.	<i>Escherichia coli</i>	12 mm	11mm	18mm	15mm	-
5.	<i>Pseudomonas aeruginosa</i>	-	11mm	17mm	12mm	13mm
6.	<i>Klebsiella pneumoniae</i>	11 mm	-	15mm	-	-
7.	<i>Candida albicans</i>	4 mm	7mm	11mm	17mm	10mm
8.	<i>Aspergillus flavus</i>	14 mm	-	12mm	14mm	13mm

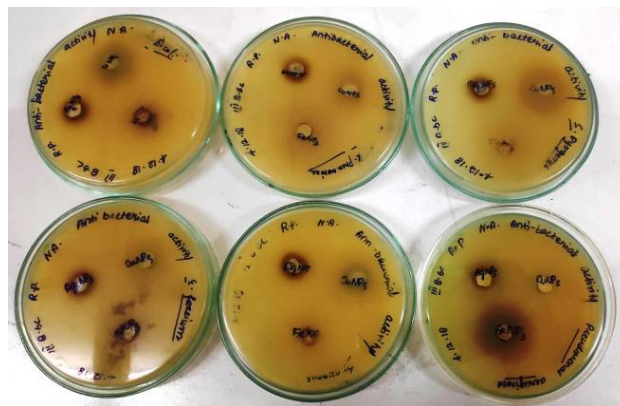


Figure: 5 Anti-microbial activity of nanoparticles against microorganisms

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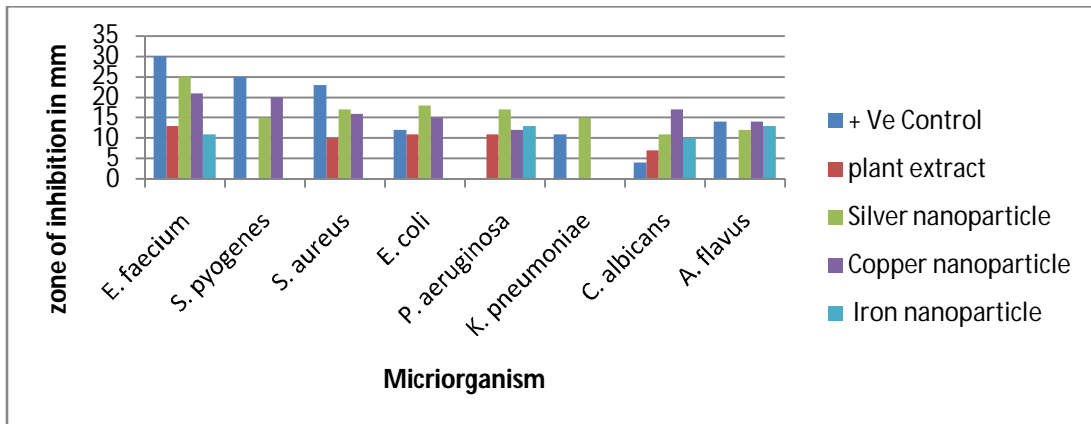


Figure: 6 Anti-microbial activity (graph)

Haemolytic activity:

The haemolytic activity was done to test the toxicity of the synthesized nano particles at five different concentrations (10, 20, 30, 40 and 50µg/ml). The study reveals that all three nano particles show relatively less haemolysis in the lower concentrations of about, 1 to 25µg/ml. and the toxicity of the nano particles increases with increase in the concentration above µg/ml with the iron nanoparticles showing highest rate of haemolysis at the rate of 89.94% in the concentration of 50µg/ml followed by silver with 86.2% and copper at 70.91%. The haemolytic activity of the nanoparticles synthesized from the leaf extract is given in the table-3, figure: 7.

Table -3

Haemolytic activity of the nanoparticles synthesized from the leaf extract of *Azadirachta indica*

Concentrations µg/ml	% Haemolysis		
	Silver nanoparticle	Copper nanoparticle	Iron nanoparticle
10	12.92	11.7	14.1
20	60.24	58.4	63.81
30	76.44	72.58	79.89
40	81.68	69.9	87.53
50	86.2	70.91	89.94

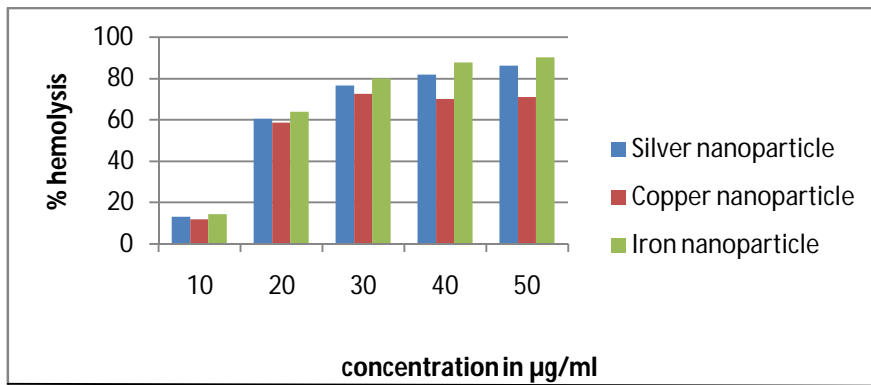


Figure: 7 Haemolyticactivity

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Genotoxicity

The result of induced nanoparticles on the root growth of *Allium cepa* show no morphological deformities found. The roots were whitish in colour, unbroken and straight. The 1% concentration of the nano particles solution show the highest number of root growths next to the control and the 3% concentration was next highest to the 1% concentration of nano particles solution. The 5% concentration of the nano particles solution shows least number of root tips in silver and copper and in iron no root tips were formed. They were stained and were observed under microscope for cell morphology (figure: 8) and any aberration in the chromosomes and to observe mitosis. The cell counting was done and the number of dividing cells and aberrant cells were counted and the mitotic index was established. In all the nano particles, the genotoxicity increases with concentration and in the 5% concentration of the iron, there was no root tip at all which showed the maximum toxicity.

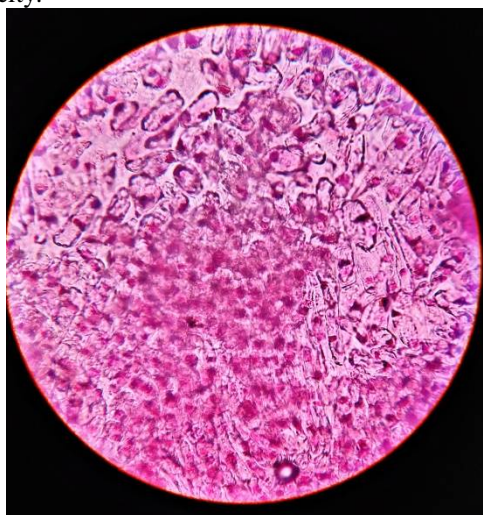


Figure: 8 Stained onion root tip under microscope (400X)

Biosensing assay:

The results of biosensing activity are as following, the results show that the silver and iron nanoparticles are positive for all the five heavy metal salts Mercuric chloride, ferrous sulphate, ferric chloride, lead acetate and zinc sulphate as there is a formation of precipitate and the copper nano particles show positive biosensing against Mercuric chloride, ferrous sulphate, lead acetate and zinc sulphate and was negative against ferric chloride as there was no formation of a precipitate or a colour change (figure: 9).

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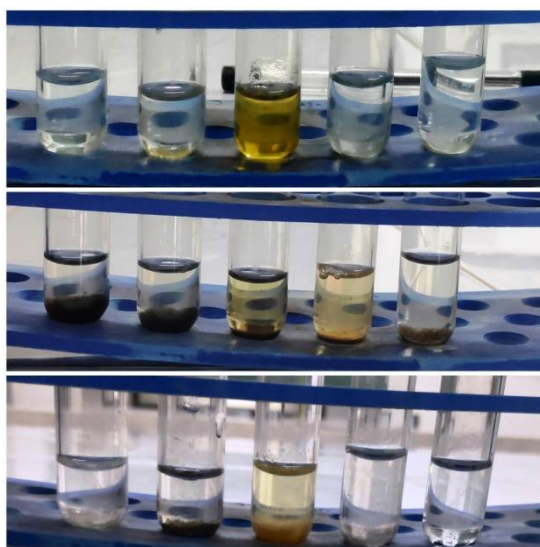


Figure: 9 Biosensing activity of silver, copper and iron nanoparticles

IV.CONCLUSION

Plants constitute important alternatives in many countries. It has also now used in the synthesis of various nanoparticles because of its various bioactive compounds and also the capacity to reduce the metal ions in a much faster rate and also economically beneficial. This study concluded the use of synthesized nanoparticles to be used in various applications especially as antimicrobial agents among which the silver nanoparticles showed highest inhibition and also was against all the microbial culture. Haemolytic activity was done to detect the toxicity of the synthesized nanoparticle and it clearly shows that the nano particles of concentration between 1- 25 μ g/ml were nontoxic and the toxicity increases with the concentration above 25 μ g/ml. the genotoxicity study shows that all the concentration of all samples except iron (5%) were not genotoxic. The biosensing activity shows that these nanoparticles are a good source for the biosensing of toxic heavy salts.

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