Research Paper
Impact Factor: 3.567
Peer Reviewed Monthly Journal
www.ijmdrr.com

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

GREEN SYNTHESIS AND CHARACTERISATION OF GOLDNANOPARTICLES USING MORINDA CITRIFOLIA LEAF EXTRACT AND ITS APPLICATIONS

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Abstract
The process of development of reliable and eco-friendly metallic nanoparticles is an important step in the field of nanotechnology. In the present work, extracellular biosynthesis of gold nanoparticles using Morinda citrifolia has been attempted and achieved rapid formation of gold nanoparticles in a short duration. X- ray diffraction (XRD), Fourier Transform Infra-red Spectroscopy (FTIR), Ultraviolet-Visible Spectroscopy (UV), Scanning Electron Microscope (SEM), Thermogravimetric Analaysis (TGA), Antibacterial and Antioxidant property were performed to characterize the formation of gold nanoparticles. The XRD peaks at 38°, 44°, 64° and 77° can be indexed to the (111), (200), (220) and (311) Bragg's reflections of cubic structure of metallic gold. The FTIR measurements showed the gold nanoparticles contains of phenolic group, amide-I, proteins compounds indicating a possible role of biomolecules responsible for capping and efficient stabilization. The synthesized gold nanoparticles were characterized by a peak at 596 nm in the UV-Vis spectrum. SEM images clearly revealed the spherical morphology of AuNPs.The thermal behavior of the crystal has been investigated by TGA. The gold nanoparticles were tested against gram-positive and gram-negative bacteria such as Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa, displayed significant antibacterial activity. The gold nanoparticles also exhibited significantantioxidant and radical scavenging activities. This environment-friendly method of biological gold nanoparticle synthesis can be applied potentially in various products that directly come in contact with the human body, such as cosmetics, foods and consumer goods, besides medical applications.

1. Introduction
Nanotechnology refers to the technology of rearranging and processing of atoms and molecules to fabricate materials to nano specifications such as nanometers. Nanotechnology is technology to manipulate and control a substance at the nanometer level. Therefore, the downsizing to the nanometer level can provide us not only the miniatures but also completely new devices operated by such special properties [1]. Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. Nanomaterials are of interest because at this scale unique optical, magnetic, electrical, and other properties emerge. These emergent properties have the potential for great impacts in electronics, medicine, and other fields. Nanomaterials have a much greater surface area to volume ratio than their conventional forms, which can lead to greater chemical reactivity and affect their strength. Also at the nano scale, quantum effects can became much more important in determining the material properties and characteristics, leading to novel optical, electrical and magnetic behavior. Due to major advances in nanomaterials characterization techniques it is becoming possible to better assess all nanomaterial types and their origins can be studied of nanomaterials in earth systems and to better frame their longer term effects impact on the earth environmental and on human health can be estimated [2,3]. Specifically gold, silver, zinc etc. nanomaterials possess unique physicochemical properties which gain a great deal of attention in biomedical applications, while platinum in energy storage.

Research interest in biocompatible gold nanoparticles has been highly increased in recent years for potential applications in nanomedicine due to theirfascinating size dependent chemical, electronic and optical properties. Some of its relevant applications like photothermal therapy, drug delivery, photodynamic therapy, gene therapy, biolabeling, biosensing, etc., are revolutionizing the field of biomedicine that attracts enormous research attention. Au nanoparticles are non- cytotoxic in nature with an additional advantage of a huge surface area, which makes their surfaces accessible for modification with targeting molecules, which make them advantageous over other nanoparticles for various biomedical applications. Gold nanoparticles are extensively used in biosensing applications these days. Determination of blood glucose level, detection of bacteria, viruses, detection of pollutants and monitoring pathogens can be effectively carried out by biosensing.

Suman, T. Y., et al.[4] described the synthesis of gold nanoparticles using an aqueous root extract of Morinda citrifolia. UV–vis spectroscopy, XRD, FTIR, FE-SEM, EDX and TEM were performed to characterize the formation of gold nanoparticles. The synthesized gold nanoparticles were characterized by a peak at 540nm in the UV–vis spectrum. The XRD peaks at 38°, 44°, 64° and 77° can be indexed to the (111), (200), (220) and (311) Bragg's reflections of cubic structure of metallic gold, respectively. The FTIR result showed that extract containing protein might be responsible for the formation of the nanoparticles and may play an important role in the stabilization of the formed nanoparticles. FESEM images revealed that the particles were triangle and mostly spherical in shape. TEM images clearly revealed the size of the nanoparticles were 12.17-38.26 nm in size [4].

2. Experimental Section

Morinda citrifolia L. has been recognized as an important herb for treating various physiological disorders worldwide. M.citrifolia is commonly known as Indian mulberry or Noni in India. Noni plant exhibits a remarkably high therapeutic and safety profile that makes it popular as a health enhancer and food supplement worldwide. The fruit contains hydrophilic compounds like carbohydrates, proteins, minerals, vitamins and small amount of fat. Fresh leaves of M.citrifolia were collected from the area of around salem district, Tamilnadu, India. The leaves were thoroughly washed with tap water and again washed with double distilled water. Cleaned leaves were dried with absorbent paper. Then 30 grams of leaves were taken and cut into small pieces. The leaves boiled for 30 minutes and filtered through filter paper. The 10 ml of leaf extract is dispensed in 100 ml of double distilled water and stirred it for nanoparticle synthesis. $2x10^{-2}$mol aqueous solution of chloroauric was prepared by dissolving 0.2 grams of chloroauric acid in 50ml of double distilled water and stirred for 30 minutes. Then 50 ml of stirred chloroauric solution was added to 100 ml of leaf extract and then stirred. Sodium hydroxide solution was added to mixture solution drop by drop to speed up the reaction. The mixture solution was left on constant magnetic stirring was continued for another one hour till nanoparticles were formed. The dried nanoparticles are grinded into fine nano powders using a mortor. This resulting powdered nanoparticle were used for characterization of gold nanoparticles.

3. Result and Discussion

The synthesized gold nanoparticles using the leaf extract of Morinda citrifolia are analysed by various characterization techniques and their results are interpreted. By using this interpreted result, various parameters of the synthesized particles are determined.

3.1 X-Ray Diffraction Analysis

X-Ray diffraction analysis is used to confirm the crystalline nature of the particle. The particle size and

structural properties of gold nanoparticles are revealed by using X-ray diffraction. Fig 1 shows that XRD pattern of gold nanoparticles. The XRD diffraction spectrum of green synthesized gold nanoparticles exhibited peaks at 2θ (38.25°, 44.40°, 64.65°, and 77.66°) were indexed to hkl values are (111), (200), (220), and (311) respectively [5]. A few intense additional and yet assigned peaks are also noticed in the vicinity of characteristic peaks are 27.1°, 32.5°, 45.4° and66.1°. These sharp peaks might have resulted from bio-organic compounds such as Amino acids/ proteins in the nanoparticle during the synthesis. All the diffraction peaks are indexed to the Face Centered Cubic (FCC) structure and is compared with Join Committee for Powder Diffraction Standards, (JCPDS) File No . 65-2870.

The narrow and strong diffraction peaks indicate the product has well crystalline in nature [6]. Table 1 shows that particle size and hkl value of observedcrystalline peaks. The XRD pattern reveals a strong plane of gold nanoparticles at plane. The particle size of AuNPs can be calculated by using Debye Scherrer's formula.

$$D = \frac{K\lambda}{\beta cos\theta} \text{ nm}$$

Where,
D = Particle size
K = Scherer's constant (0.9)
λ = Wavelength of X-ray (1.54 x 10^{-10} m)
β = Full Width Half Maximum.θ = Bragg angle
The average crystallite size of gold nanoparticle is found to be at 20.9021 nm. This study reveals that the synthesized AuNPs are of pure crystalline gold [7].

Table 1 Particle size and HKL value of observed crystalline peaks

S.No	2θ Degree	d A°	FWHM (radian)	hkl values	CrystallineSize (nm)	Averagecrystal size (nm)
1	38.253	2.3509	0.5685	111	25.8044	20.9021
2	44.407	2.0384	0.8694	200	17.2188	
3	64.652	1.4405	0.7689	220	21.3318	
4	77.664	1.2285	0.9241	311	19.2535	

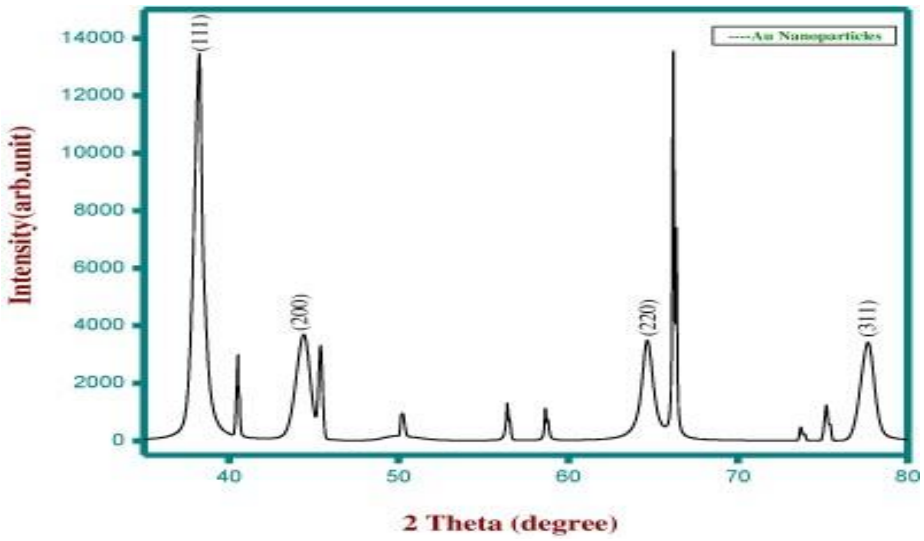


Fig 1 XRD pattern of gold nanoparticles

3.2 Fourier Transform Infrared Spectral Analysis (Ftir)

FTIR measurement is carried out to identify the possible biomolecules responsible for capping and efficient stabilization of gold nanoparticles synthesized using Morinda Citrifolia leaf extract [5]. Table 2 Vinbrational assignments of gold nanoparticles. Fig 2 shows that FTIR spectrum of gold nanoparticles. The FTIR spectrum of gold nanoparticles shows a very strong peak at 3427 cm^{-1} which is assigned as NH stretching vibration of amide (II) band. The peak is observed around 2922 cm^{-1} and 2841 cm^{-1} that are assigned to the C-H stretching vibration of alkyl and aldehyde groups. The very strong absorption band at 1638 cm^{-1} is identified as the amide I and arises due to the carbonyl stretching vibration in the amide linkages of proteins. The presence of the amide linkage suggests that there are some proteins in the reaction mixture [6]. This protein might be responsible for the formation of nanoparticles andmay play an important role in the stabilization of the formed nanoparticles. The band located at 1385 cm^{-1} and 1040 cm^{-1} is due to C-N stretching vibrations of aromatic and aliphatic amines, respectively [7]. A peak is observed around 608 cm^{-1} that indicates the presence of alkyl halides. It is a very weak band and it indicates the presence of gold nanoparticles [8].

The bonds or functional groups such as NH, C-H, C=O, C=C, C-N bonds are derived from water soluble compounds in Morinda citrifolia leaf. Therefore, it may be assumed that water soluble compounds such as flavoinds, proteins , amine and saponins are the capping ligands of the nanoparticles [9].

Table 2 Vibrational assignments of gold nanoparticle

S.NO	Wave number (cm^{-1})	Type of vibrations	Intensity
1	3427	NH stretching	Strong
2	2922 and 2841	C-H stretching	Medium

3	1638	C=O and C=C stretching	Medium
4	1385 and 1040	C-N stretching	Medium
5	608	Presence of gold nanoparticles	Weak

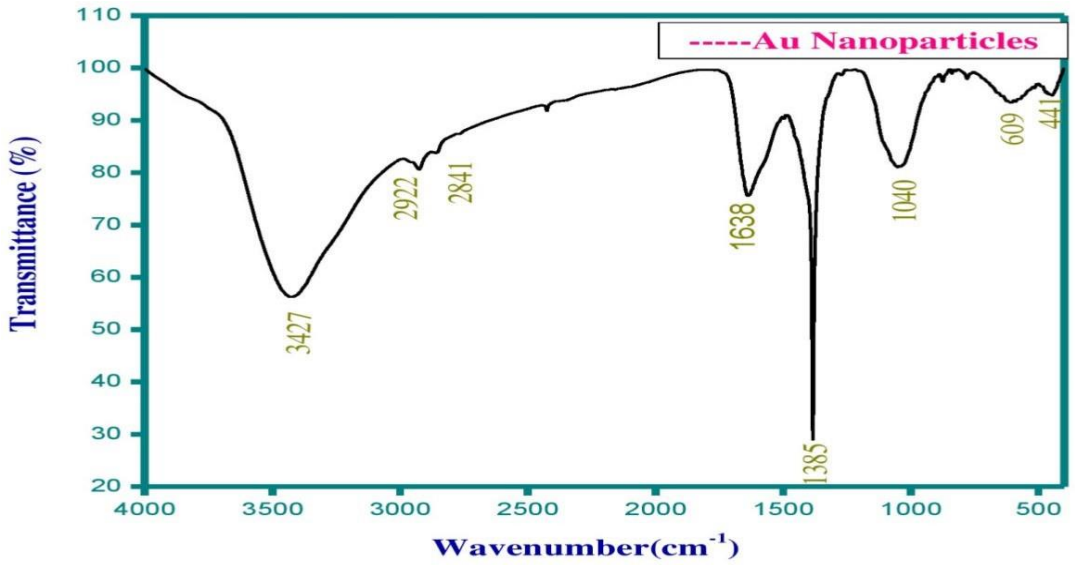


Fig 2 FTIR Spectrum of Gold Nanoparticles

3.3 Uv-Visible Spectral Analysis

UV-Visible spectroscopy is an important technique to determine the morphology and stability of nanoparticles. The formation of gold nanoparticles was observed with change of colour from pale yellow to dark violet which is shown in Fig 3**.** Which is the characteristic for gold nanoparticles formation due to excitation of surface plasma resonanonce (SPR) and reduction of $AuCl4^-$ [8]. The formation of gold nanoparticles in aqueous solution were confirmed by using UV-Visible spectral analysis in the range from 500 to 600 nm [10].

The band gap energy due to energy transfer spectra is calculated using formula

$$E = \frac{hc}{\lambda} \text{ eV}$$

Where,

h = Planck's constant (Joule sec)C = Velocity of light (m/sec)

λ = Wavelength of light (nm)

The UV-Visible Spectrum is recorded for gold nanoparticles using Morinda citrifolia leaf extract**.** Fig 4 shows that UV-Vis Absorbance spectrum of gold nanoparticles. The result showed optical absorbance

peak at about 597.55 nm attributed to the Surface Plasmon Resonance (SPR) [6] and band gap energy is 2.1eV which are given in Table 3. The band gap energy is evident for the presence of gold nanoparticles.

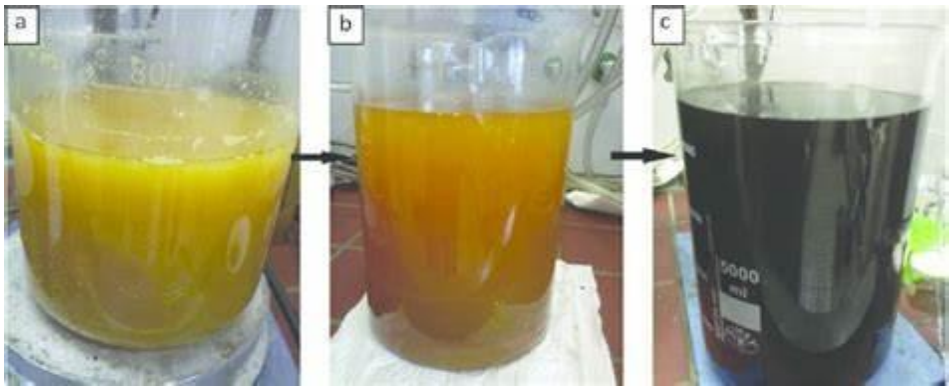


Fig 3 Colour change of gold AuNPs from pale yellow to dark violet colour

Table 3 UV-Visible Absorbance Spectrum of Gold nanoparticles

Leaf	ColourChange	Peak Observed	Band gap energy
Morindacitrifolia	Pale yellow to darkviolet	597.55	2.1 ev

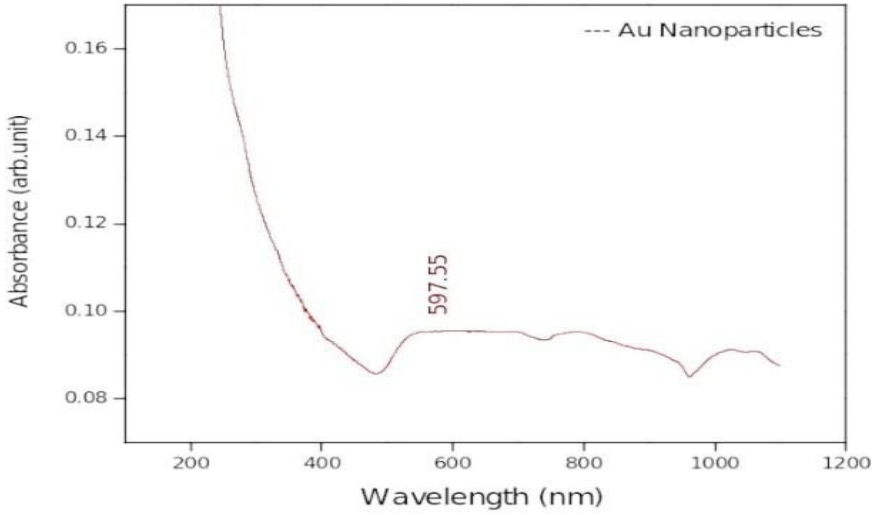


Fig 4 UV-Visible Absorbance spectrum of Gold Nanoparticles

3.4 SEM Analysis

The surface morphology, size and shape of gold nanoparticles were analysed by Scanning Electron Microscope. The Fig 5 shows that morphology of synthesized gold nanoparticles of Morinda citrifolia leaf extract. Predominantly the shapes of the particle are Spherical and aggregated into large particles. A closer look shows the presence of several nanoparticles aggregates and some individual crystals are clearly visible [9].

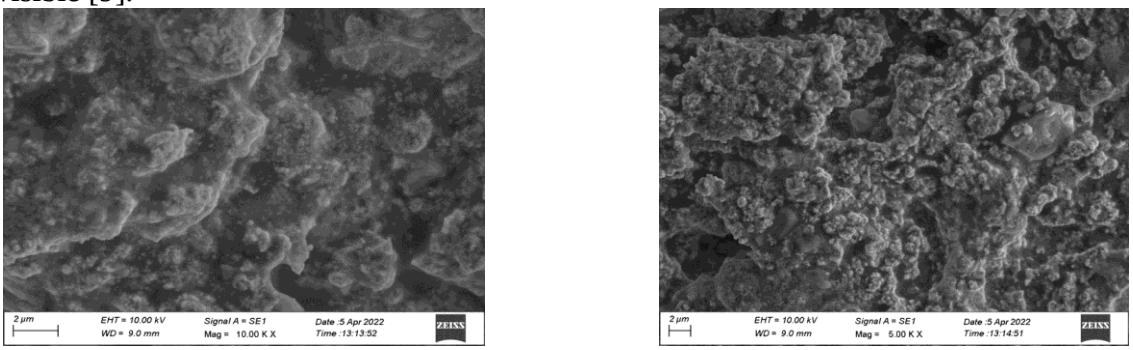


Fig 5 SEM images of Gold Nanoparticle

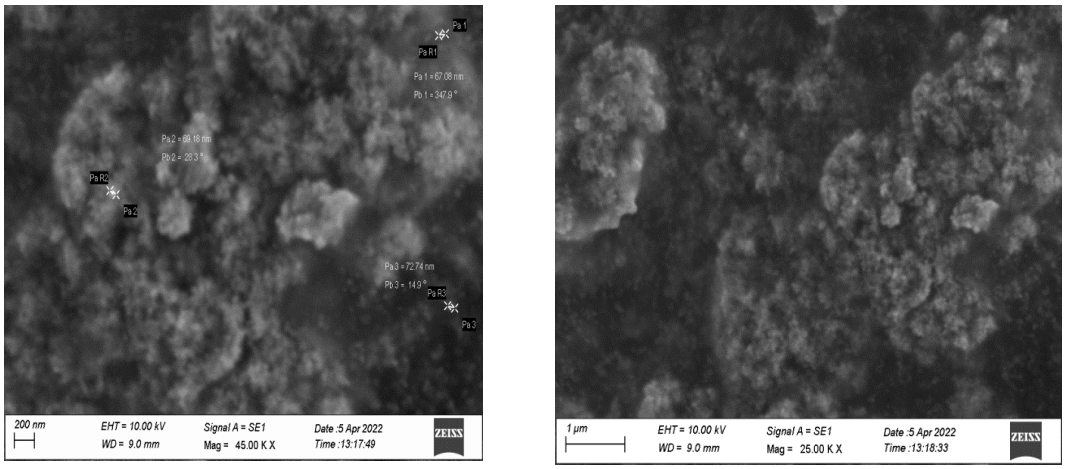


Fig 6 SEM image showing surface size of Gold nanoparticles

Fig 6 shows that SEM image showing surface size of gold nanoparticle. SEM analysis is verified the presence of gold nanoparticles. SEM reveals the morphological structure of nanoparticles. SEM images are seen in different magnification ranges and they are in spherically shaped nanoparticles.

3.5 Thermo gravimetric Analysis (TGA)

The thermal stability of the AuNPs was studied using TGA (SDT Q600). TGA analysis was used to determine the total amount of phytochemical residuals that capped the AuNPs ranging from phenolic compounds and small proteins that might be present in the plant extract and are adsorbed on the nanoparticle surface. Following the purification methods, impurities with in the sample could be eliminated [11]. For this purpose 0.51247 mg of AuNPs was taken in alumina crucible. Nitrogen gas is allowed inside the furnace to maintain a constant inert atmosphere for the entire experiment. The

AuNPs was heated in the range of 30℃ to 800℃ with the heating rate of 5℃ min^{-1}. It provides reliable information about the mass and energy changes of the sample with respect to the increasing temperature. The TGA graph is shown in Fig 7.

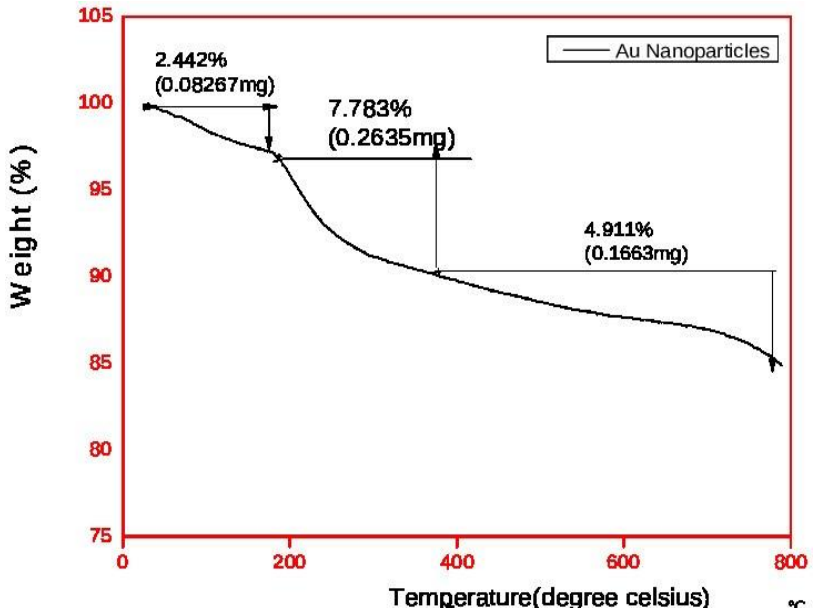


Fig 7 Thermo gravimetric analysis of gold nanoparticles

The AuNPs compound exhibits three stage weight loss, first stage starting from 40℃ to 175℃ with the mass loss of almost 2.442% (0.08267 mg). The second stage weight loss indicate the decomposition from 175℃ to 390℃ with the mass loss of almost 7.783% (0.2635 mg). The third stage weight loss indicate the decomposition from 390℃ to 780℃ with the mass loss of almost 4.911% (0.1663 mg) of the synthesized material. The remaining 84.86% of organic components of AuNPs were degraded, suggesting that biological ingredients from the plant extract capped the AuNPs surface [12].

3.6 Phytochemical Constituent for Morinda Citrifolia

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and and other animals. It revealed the presence of alkaloids, steroids, saponins, amino acids, anthraquinones and tannins. Quantitative determination of total phenolics, total flavonoids, and various in vitro antioxidant activities of methanolic extract was carried out to determine calorimetric method [13]. Table 4 shows that phytochemical test of leaf extract.

Table 4 Phytochemical test of leaf extract

S.No	Phytochemicals Test	Morinda Citrifolia
	Solvent extract	**Water**
1	Alkaloids Dragendorff's Test	–
2	Flavonoids NaOH	+
3	Saponins Foam formation	+
4	Proteins Biuret test	–
5	Amino acids Nitric acid	+
6	Phenols Ferric chloride test	+
7	Steriods Liberman Burchard test	+
8	Anthraquinones Borntrager's test	–
9	Tannins Ferric chloride	+

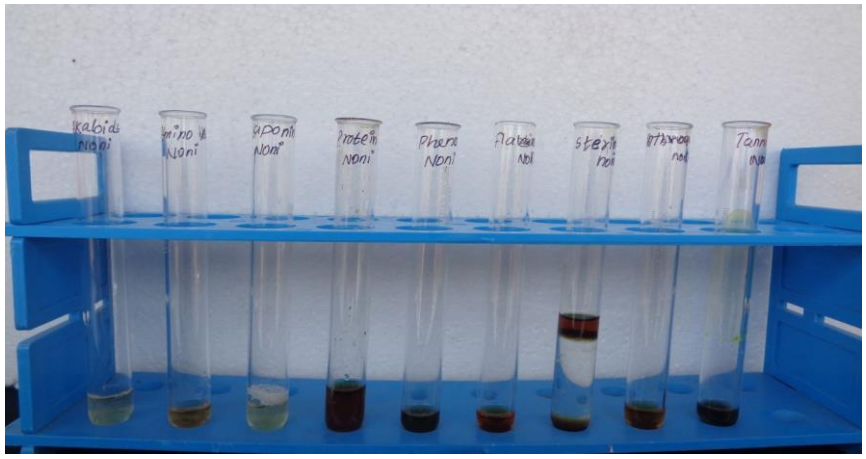
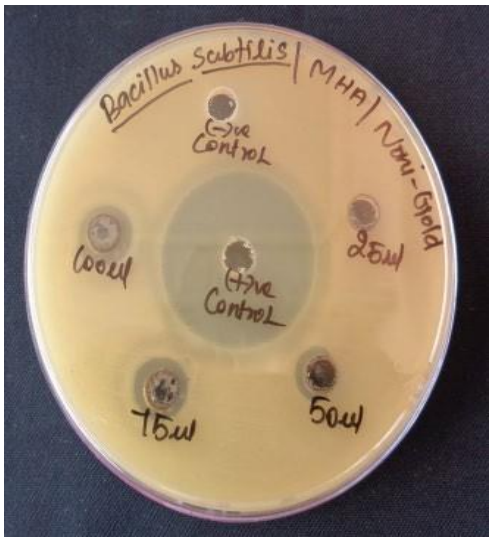


Fig 8 Phytochemical Screening

Fig 8 shows that phytochemical screening. The plant extracts was screened for the presence of various secondary metabolies such as Alkanoids, Flavonoids, Saponins, Proteins, Amino acids, Phenols, Steriods, Anthraquinones, Tannis. The medicinal value of Morinda Citrifolia can be correlated due to the presence of variousbioactive chemical constituents.

3.7 Antibacterial Activity

Antibacterial activity of green synthesized gold Nanoparticles is examined using disc diffusion method. The bacterial activity of biosynthesized gold nanoparticles using leaf extract Morinda citrifolia has potential antibacterial activity against both Gram-positive and Gram-negative human pathogens which is given in Table 5. AuNPs displayed Antibacterial activity against Gram positive and Gram negative bacteria, with varying concentration as suggested by the diameter of inhibition zone [14,15]. The antibacterial activity of gold nanoparticles at varying concentration (25µl, 50µl, 75µl and 100µl) is shown in Fig 9. The zone of inhibition is measured against two gram positive bacteria such as Bacillus subtilis and Staphylococcus aureus, Gram negative bacteria such as Pseudomonas aeruginosa. At higher concentration the maximum zone of inhibition is observed against gram-negative bacterium (Pseudomonas aeruginosa) and the minimum zone of inhibition are observed against gram-positive bacterium (Bacillius subtilis**).** Fig 10 clearly shows the maximum and minimum zone of inhibition of gold nanoparticles. Several researches have confirmed that antimicrobial activity of gold nanoparticles against the food related bacteria Bacillus subtilis, and Staphylococcus aureus and compared with reported work (Russell and Hugo, 1994 and Ip et al., 2006) [16]. This study also suggests that green synthesized gold nanoparticles can be used as an alternative to existing antimicrobial agents [17].



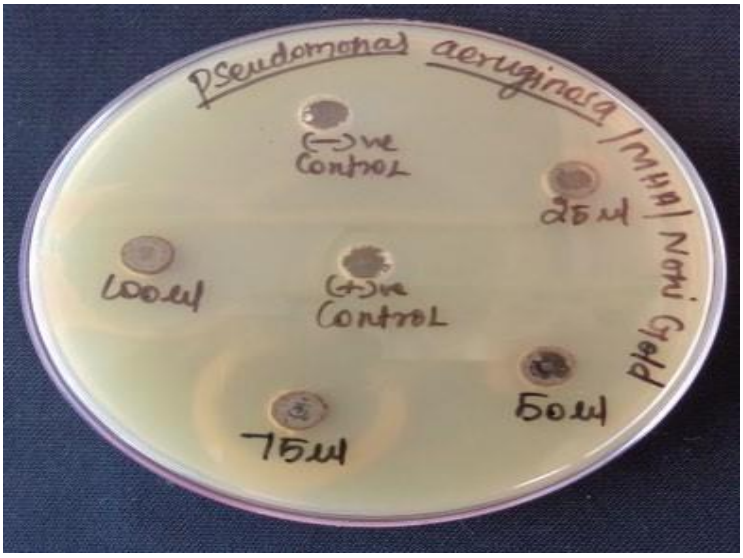


Fig 9 Antibacterial activity of Gold nanoparticle

But the present study illustrates good zone of inhibition against all pathogenic bacterial strains. It shows that, increased zone of inhibition were observed at higher concentration of gold nanoparticles. So, the zone of inhibition is directly proportionalto the concentration of gold nanoparticles.

Table 5 Zone of inhibition of Gram positive and Gram negative bacteria

S.no	Test organisms	Zone of inhibition			
		25 µl	50 µl	75 µl	100 µl
1	Bacillus subtilis	NA	10	11	14
2	Staphylococcusaureus	NA	NA	11	20
3	Pseudomonasaeruginosa	10	13	15	22

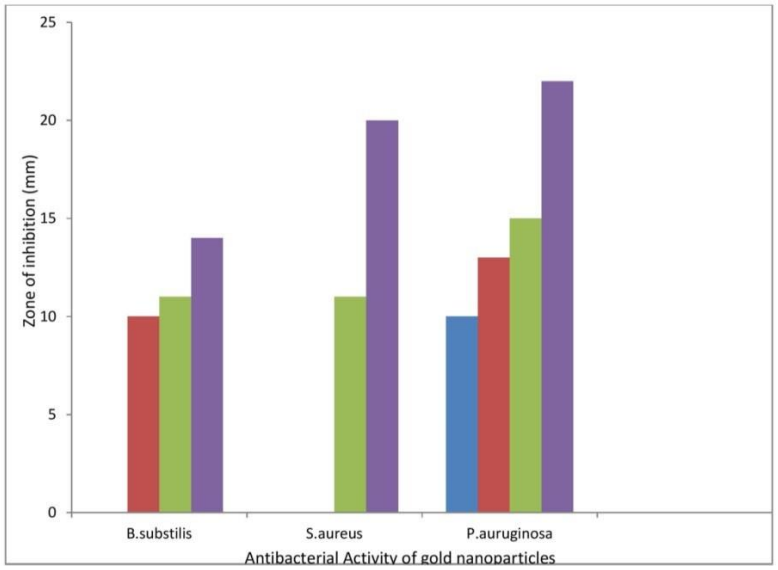


Fig 10 Zone of inhibition of Gram positive and Gram negative bacteria

3.8 Antioxidant Activity

Antioxidant prevents the cell damage by neutralizing the free radicals in the cellular system. The antioxidant activity is due to the phenolic groups such as flavonoids and flavonals present in the plant extracts [18]. 1,1-Diphenyl-2- picrylhydrazyl (DPPH) is a well known method to determine the antioxidant activity due to its potential on free radical's reduction. DPPH is the free radical scavenging activity of AuNPs which shows effective inhibition. The absorbance value of DPPH is 517 nm [19]. Fig 11 shows, free radical scavenging activity of AuNPs biosynthesized by leaf extract of Morinda citrifolia.

Table 6 shows that DPPH scavenging activity. The AuNPs was investigated in several concentrations (20, 40, 60, 80 and 100 µg/ml). DPPH scavenging effect wasobserved from 42.02% at a concentration of 20 µg/ml to 62.97% at a concentration of 100 µg/ml of AuNPs. The results revealed that radical scavenging activity of gold nanoparticles is increased by increasing the concentration [20].The IC50 value of synthesized AuNPs is calculated as 24.65 µg/ml**.** Fig 12 shows the scavenging capacity of AuNPs on DPPH free radicals.

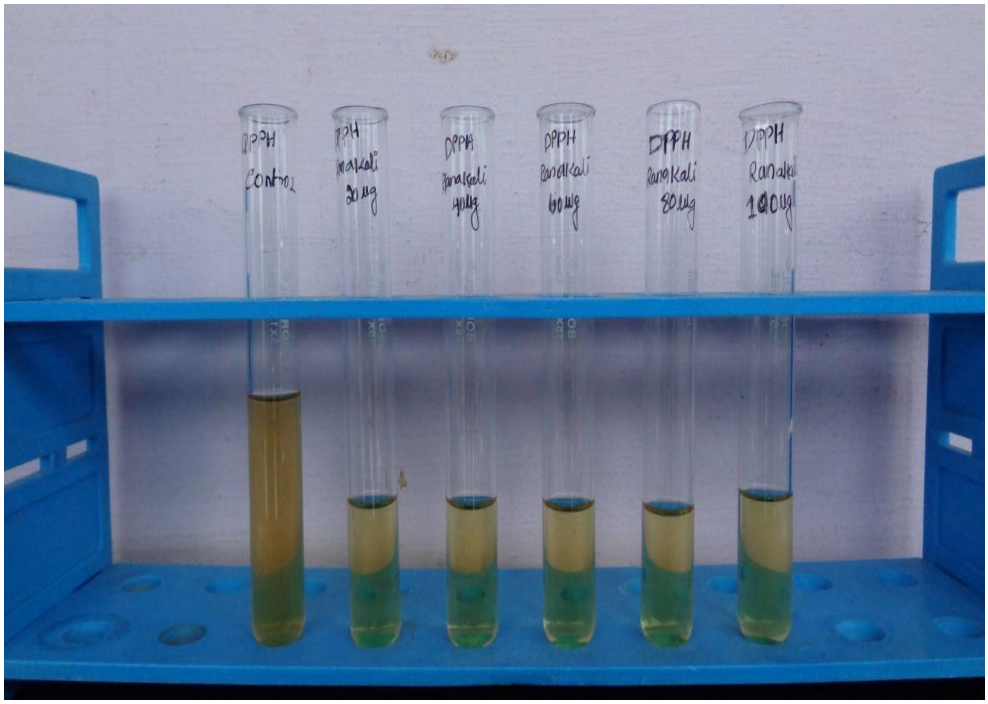


Fig 11 Antioxidant activity of Gold nanoparticle

Table 6 DPPH Scavenging Activity

S.No	Concentration(µg/ml)	Antioxidant activity % of inhibition	IC 50 value
1	20	42.02	24.65
2	40	49.87	
3	60	51.69	
4	80	56.52	
5	100	62.97	

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

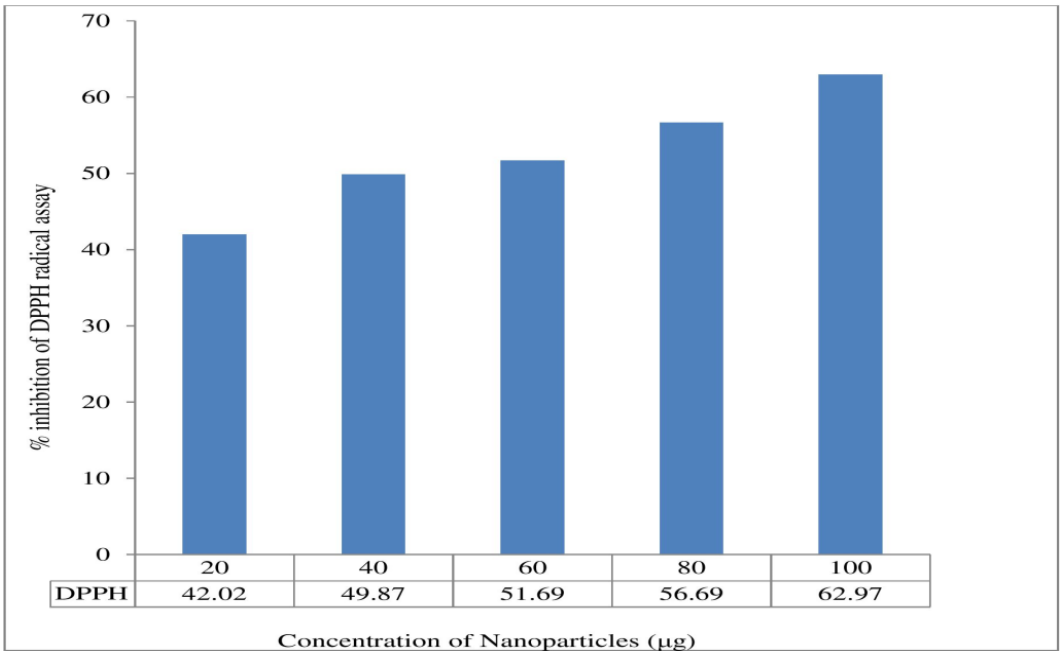


Fig 12 Scavenging capacity of Au-NPs on DPPH free radicals

Conclusion

Green synthesis of gold nanoparticles used in this experiment is found to be eco-friendly, non-toxic and less usage of chemicals when compared to physical and chemical methods. The XRD analysis determines the particle size is about 20.9021nmproved that the sample gold nanoparticles is crystalline in nature. In FTIR, the presence of functional groups in gold nanoparticles is determined such as phenolic group, primary amines, proteins, aromatic compounds and steroids. UV-Visible absorption spectrum reveals that a gold nanoparticle shows the absorbance peak is obtained at 597.55 nm and its band gap is calculated as 2.1 eV. The SEM analysis of gold nanoparticles is demonstrated the surface morphology and the particle is spherical shape in structure.TGA studies indicate that the compound is thermally stable upto 800℃. The anti-bacterial activity of gold nanoparticles has proved that it can be used as potent anti-bacterial agent. Further, DPPH assay indicated gold nanoparticles has antioxidant activity with IC50 value of 24.65 µg/ mL.

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