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Biosynthesis and characterization of gold nanoparticles by *Myristica dactyloides* Gaertn for antimicrobial activity

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Abstract
Nanotechnology deals with nanoparticles with sizes ranging from 1-100 nm that are purposely used in healing contact, atomic flow, and other areas. The influence of nanoparticles on the natural environment is causing increasing concern among natural scientists and the general public. Gold nanoparticles are frequently made from compounds that are hazardous and flammable in some way. This review focuses on a biosynthetic and ecologically friendly approach for pharmacological applications of gold nanoparticles derived from Myristica dactyloides fluid extract. UV-Vis spectrometry, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) with Energy-Dispersive Spectroscopy (EDX), Dynamic Light Scattering analyses (Particle size and Zeta potential), and X-ray Diffraction have all confirmed the arrangement and characterization of AuNPs (XRD). The antibacterial properties had worn off on a few human pathogenic strains at various doses. The Au nanoparticles successfully inhibited the growth of microbial pathogens in a time-dependent and backbreaker manner.

Keywords: medinilla beddomei, phytochemicals, GC MS analysis, acetone extract, fever

Introduction
Nanotechnology is a really capable innovation; nanotechnology is one of the most in-demand areas of inquiry within the sophisticated think about of texture science. Nanoparticles appear to be underutilized or progressed forward in terms of qualities such as particle size, dispersion, and shape, among others. Novel uses of nanoparticles and nanomaterial's are rapidly emerging in a variety of fields [1]. This provides a significant opportunity for the development and invention of a wide range of novel products, with potential therapeutic uses in early disease detection, treatment, and prediction. Organic atoms are well-suited for nanotechnology applications due to their superior characteristics. The organic particles are subjected to a highly controlled assembly process to make them suitable for the solid and environmentally safe metal nanoparticles union [2]. Due to their one-of-a-kind chemical, optical, attractive, mechanical, and electric appealing qualities, metal nanoparticles have gotten a lot of attention. The combination of metal and semiconductor nanoparticles might be a huge area of research because of the possible uses, which have resulted in the development of innovative breakthroughs [3]. Gold nanoparticles provide a distinct class of biocompatible vectors capable of upholding this promise through the precise cell and atomic targeting, which can provide previously untapped means for situation-specific diagnosis and treatment of therapeutic diseases [4]. This study examines the technique for conjugating AuNps with certain biomolecules, as well as the subtle aspects that arise from pondering the target selectivity and cytotoxicity effects of such conjugated gold nanoparticles.

Materials and Methods
Sample collection
Between November and December 2021, the plant Myristica dactyloides was harvested in the Tiruchirappalli district of Tamil Nadu, India. (Figure.1)

Aqueous Extraction
The plant parts were collected individually, rinsed three times with purified water, shade-dried for five days, and pounded into a fine powder. The fine powder of the plant fabric was weighed after sterilisation at 121°C for 15 minutes. 20 g of sterile fine powder was mixed with 200 ml Milli Q water and kept for 10 minutes in a bubbling water shower at 100°C. The extracts were filtered on Whatman 1 channel paper and stored in the fridge at 4°C for advanced processing to avoid microbial contamination.

Fabricating of gold nanoparticles
The gold chloride was mixed with pre-sterilized Milli Q water at a concentration of 10^{-3} M. For the synthesis of gold nanoparticles, 10 ml of plant extract was mixed with 90 ml of 10^{-3} M gold chloride. Without incorporating

plant extracts, gold chloride was taken in comparable proportions to primary individual controls. The saline bottles were firmly secured with aluminium foil in order to keep a strategic distance from photo decreasing of gold particles, and they were brooded at room temperature under dim light.

Characterization of nanoparticles

After AuNPs, the arrangement was characterised by UV-Vis spectrometry, Fourier Change Infrared Spectroscopy (FTIR), Checking Electron Magnifying instrument (SEM) with Energy-Dispersive Spectroscopy (EDX), Field Emanation Filtering Electron Magnifying lens (FESEM), Transmission electron magnifying lens (TEM), Energetic Light Diffusing exams (Molecule measure and Zeta potential), and X-ray Diffraction (XRD) [5].

UV-VIS spectroscopy

To determine the active conduct of Au nanoparticles, a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19, was used to characterise them. The testing ranged from 200 to 800 nm, with a check speed of 480 mm/min. The spectrophotometer's standard rectification has been eliminated by using a white character.

Fourier transforms-infra red (FT-IR) spectrometry

FT-IR was used to measure the investigation of the bio-reducing specialists found in each of the extracts. A small aliquot of the concentrated response blend was tested in the transmittance mode at 400 to 4000 cm-1 after the response. After dissecting the biogenesis of nanoparticles, the spectra of the extract were collected.

Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

The Joel JSM-6480 LV SEM machine was used to characterise the cruel molecular estimation and morphology of nanoparticles in this investigation. The vitality dispersive X-ray spectrometry (EDS) along with the SEM was used to examine the test's composition. The SEM (JEOLJSM 5800) machine was used to investigate the EDS of the Ag test. The EDS frequently reveals the proximity of phases.

Dynamic Light Scattering analyses *(Particle size and Zeta potential)*

The Au powder has been dispersed in water by a horn-type ultrasonic processor [Vibronics, show VPLP1] in parliamentary legislation to urge out the molecular estimate dispersion. The normal molecule estimation of gold nanoparticles was studied using energetic light scrambling (DLS), which is based on the laser diffraction strategy and employs a variety of scrambling methods. The planned test has been dispersed in deionized water, followed by ultrasonication. The supernatant has gathered after the arrangement has been filtered and centrifuged for 15 minutes at 250C at 5000 rpm. To eliminate molecular measure scattering, the supernatant was weakened 4 to 5 times before being evaluated on a computer-controlled molecule size analyzer (ZETA Sizer Nano arrangement, Malvern instrument Nano Zs). The electrical voltage within the twofold layer of particles covering a particle at the limit of the molecule surface and the adsorbed particles within the diffuse layer is depicted by zeta potential [6]. With a Zetaphorementer IV, Zeta possibilities have been discovered (CAD, France).

X-ray diffraction method

The X-ray diffraction process (Philips Container expository, The Netherlands) used Cu radiation to examine the stage advancement of calcined powder as great as that of sintered testing. Individually, the generator voltage and current were set to 40 kV and 30 mA. The Au test was performed in continuous filter mode for the run 10.0000 - 90.0000º. The filter rate was 0.60 seconds per second.

Antimicrobial screening AuNPs

The gold nanoparticles were tested for antibacterial sensitivity against specific microbial strains (obtained from MTCC and NCIM, India) using the circular dissemination approach [7]. The test strains were *Aeromonas liliqueciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumoniae* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC (F4). The bacterial and parasite suspension on the surface of MHA and PDA agar plates was vaccinated with a sterile cotton wipe. One by one, the 15 and 30 liters of the sample-coated plate have been placed on agar plates. The sterile ponder was used as a negative control. The plates were heated to 371°C for 24–48 hours (for bacteria) and 251°C for 48–72 hours (for organisms) [8]. The zone of constraint has been measured with a ruler after hatching. The trial has been run three times and the mean values have been displayed.

Results

Biosynthesis of Au nanoparticles

The gold chloride arrangements and the plant fluid arrangements were made separately. For the union of gold nanoparticles, the plant extract was mixed with gold chloride. During this procedure, the colour changed from pale greenish to pink, indicating that gold nanoparticles were arranged in a certain pattern (Figure 2) [9].

UV-VIS spectral analysis

The researchers used UV-VIS spectroscopy to discover the proximity of facial hair crests at 540 nm (Figure 3). The absorbance maxima of the assimilation spectra of Au nanoparticles shaped within the response medium are at 540 nm [10]. The particles are polydispersed, as evidenced by a sudden expansion of the crest from 480 to 680 nm. Throughout the interim, the top became apparent and rising. This rising crest denoted the growing amalgamation of nanoparticles as time progressed. With the passage of time, the assimilation crest's attributes improve. The inflammation of the SPR within the metal nanoparticles causes this distinctive colour variation [11]. The metal particles are rapidly depleted; in less than two hours, more than 90% of the Au+ particles had been depleted. After the metal particles have expanded to the plant of your choice. 4 weeks after their merger, the metal particles were shown to be in a stable arrangement. By stability, we mean that there is no observed variation in the optical characteristics of nanoparticle arrangements across time at that point. For the sake of UV-vis information, it has been established that metal particles are reduced [12].

Fourier Transform Infra-Red (FTIR) Spectroscopy

The bioactive chemicals generated by the plant and connected to the nanoparticles were investigated using FT-IR on the manufactured gold nanoparticles. The Plant tests appear to have a few functional bonds associated with them in the FTIR images, which give them stability by capping them. 3625 cm-1, 2080 cm-1, 1644 cm-1, 1391 cm-1, and 706 cm-1 are taken from the figure. The RCH=CHR out-of-plane is compared to the 706 cm-1, the S=O Sulfate is compared to the 1391 cm-1, the C=N is compared to the 1644 cm-1, the R-N=C=S is compared to the 2080 cm-1, and the Goodness free is compared to the 3625 cm-1. As a result, proteins and metabolites with functional clusters have engulfed the produced nanoparticles (Figure 4). We discovered that the carbonyl bunches from amino corrosive buildups and proteins had a strong ability to twist metal, implying that proteins could potentially create metal nanoparticles (i.e., gold nanoparticle capping) to avoid agglomeration and therefore stabilize the medium. This shows that natural particles could potentially serve the dual purpose of organizing and stabilizing gold nanoparticles in fluid media. Flavanones or terpenoids are kept on the surface of metal nanoparticles, according to carbonyl bunches. Flavanones or terpenoids appear to be adsorbed on the surface of metal nanoparticles, possibly by interactions with carbonyl bunches of -electrons in the absence of other solid ligating Specialists. The presence of reducing sugars within the arrangement may be capable of reducing metal particles and arranging the contrasting metal nanoparticles [13]. It's also possible that terpenoids contribute to the reduction of metal particles by oxidizing aldehyde bunches within atoms to carboxylic acids. Once the different divisions of the plant extract have been isolated, recognized, and independently assessed for the diminishment of the metal particles, these difficulties can be addressed [14]. This, or even a point-by-point examination, is currently happening.

Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS)

The SEM image of gold nanoparticles synthesised by green amalgamation with 5% takes off extract and 1mM HAuCl4 concentration produced a clear image of profoundly thick gold nanoparticles. The SEM image of gold nanoparticles made from plant extract confirmed the advancement of gold nanostructures (Figure 5). The EDS examination revealed that the test has the required stage of gold (Au) and potassium (K). It has discovered the presence of pure gold nanoparticles in more parts than other agents [15]. This is most likely owing to the proximity of the substrate to which the NP test was conducted during SEM microscopy (Figure 6).

Dynamic Light Scattering of Particle Size analyzer

The molecular measurement of the Au nanoparticles experiments is shown in Figure 7. After reviewing the data, it was discovered that the dispersion of Au nanoparticles is measured by the graphical depiction of the normal molecule. They were in a 20-80 nm circle. In any event, the rate of nanoparticle display beyond 100 nm is extremely high. Within the arrangement, the highest division of Au-NPs was 266 nm. It was obvious from the plot that the arrangement consisted of nanoparticles of various sizes, which is without a doubt in agreement with the results obtained by SEM investigation [16].

Dynamic Light Scattering of Zeta Potential Measurement

Figure 8 depicts the zeta potential (), which appears to be a measure of the electrostatic potential on the nanoparticles' surfaces and is linked to the electrophoretic versatility and soundness of the nanogold nanoparticle suspension. The vivaciously direct steady was revealed by the significant absorbance of Zeta Potential. The particles experience agglomeration/conglomeration in this way, which helps to stabilise them. As a result, there were a few potential charges on the nanoparticles' surface, which made them stable. This examination provided us with the charge potential. The soundness of a form/structure has a coordinate relationship with zeta potential (surface potential).

XRD analysis

The XRD image of the test after the gold chloride hydrate expanded has been shown (Figure 9).It refers to the XRD design of the gold nanoparticles that were given. The positions of 38.1, 44.3, 64.7, and 77.5 in the crest design indicate that gold is close by and that the esteem is trustworthy. Even though Figure.7 agrees with Bragg's

reflection values of 2, the gold nanoparticles produced have a random shape. The nanoparticles appear to be crystalline in the XRD designs

Antimicrobial studies

Using the plate dissemination approach, the antibacterial mobility measure AuNPs has been tested against various NCIM and MTCC species. For microorganisms and parasites, the test concentrations (15 and 30 L/disc) deliver zone on MHA and PDA plates, respectively. Within the bacterial division, the test is particularly effective against Salmonella typhimurium NCIM 2501 (B5), with less influence on Micrococcus luteus NCIM 2871 (B4). By the by, no organisms have been observed that are effective against Trichophyton rubrum MTCC 3272 (F4) due to a smaller impact. Against specific microbes, the higher (30 L/disc) concentration had a greater zone impact than the lower (15 L/disc) concentration. When compared to the positive control, all of the microbial strains are more susceptible to the greater concentration (30 L) for the test, except B3, B4, and B6. There is no antimicrobial movement in the setup without the test used as a vehicle control (sterile triple refined water), implying that antimicrobial action is directly linked to the test (Table 1). The gold nanoparticles are not only attached at the surface of the cell membrane, but also reach the core of microscopic organisms, causing injury to the cells through their interaction with phosphorus/sulfur-containing DNA and its replication. The test has been most successful against B5 in microscopic species, while B4 has had a lesser impact. This has been found to be effective against F4 parasites, with a lesser impact on F2. All of the microbial strains were shown to be more susceptible to greater concentrations (30 L), implying that silver materials are a viable alternative to antimicrobials for treatment [17]. These nanoparticles release gold particles into bacterial cells, enhancing their bactericidal activity [18]. There is no antimicrobial movement in the arrangement without the test (sterile triple refined water), indicating that antimicrobial movement is only related to the test.

Table 1: Antimicrobial activity of AuNPs

S. No	Test Microorganisms	AuNPs µL/disc		PC	Diseases	Route of Transmission
Bacteria		15	30	10 mcg		
1.	*Aeromonas liquefaciens* (B1)	11	12	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus faecalis* (B2)	12	14	8	Endocarditis/Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* (B3)	15	22	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* (B4)	16	18	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium* (B5)	14	16	0	Typhoid	Water / Food
6.	*Vibrio cholerae (*B6)	11	14	16	Cholera	Water / Food
Fungi						
7.	*Candida albicans* (F1)	11	13	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. (F2)	11	14	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* (F3)	12	14	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* (F4)	12	13	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC -Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc); Samples – 15, 30 mg/ml (well)

Fig 1: *Myristica dactyloides Gaertn*

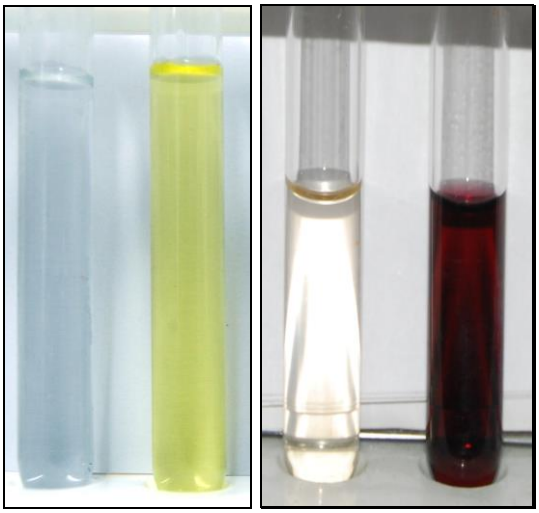


Fig 2: Plant broth and biosynthesised Gold Nanoparticles

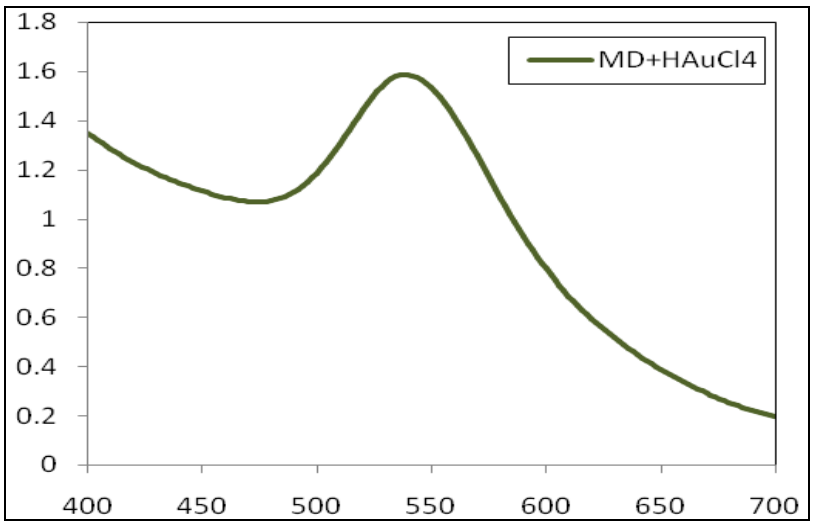


Fig 3: UV-Spectrum of AuNPs

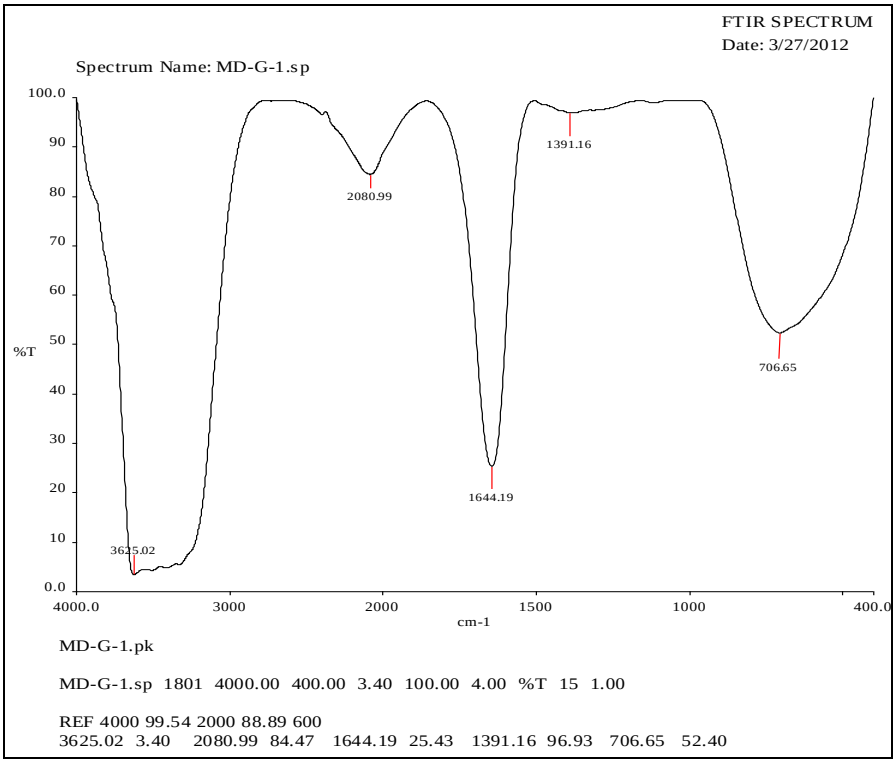


Fig 4: FTIR spectrum of AuNPs

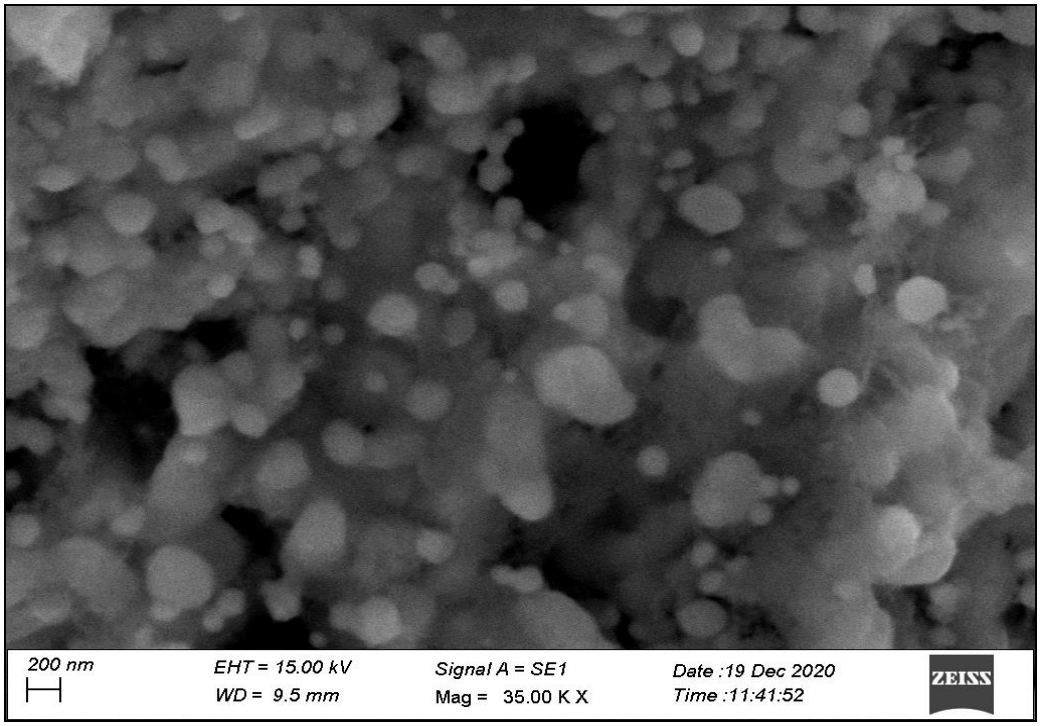


Fig 5: SEM Image of AuNPs

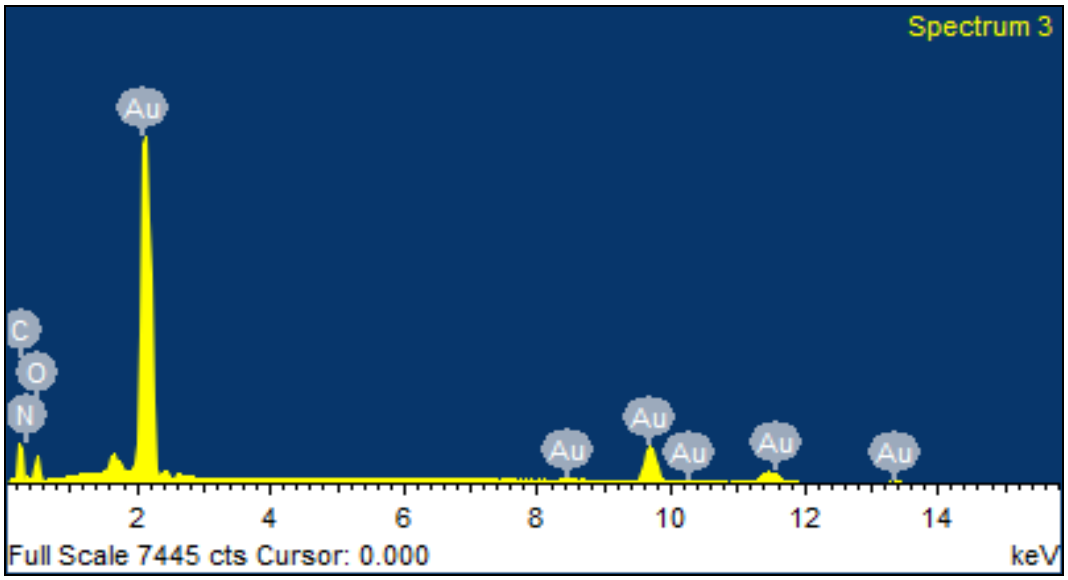
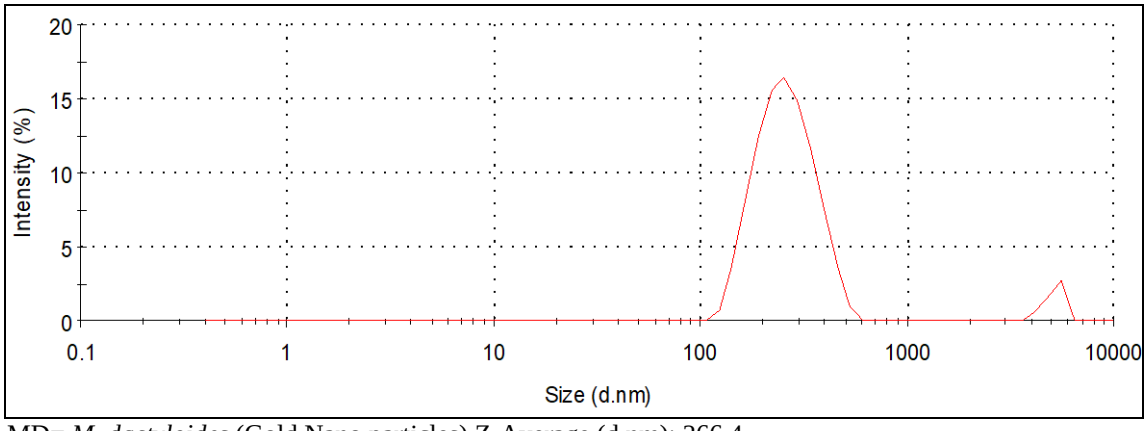
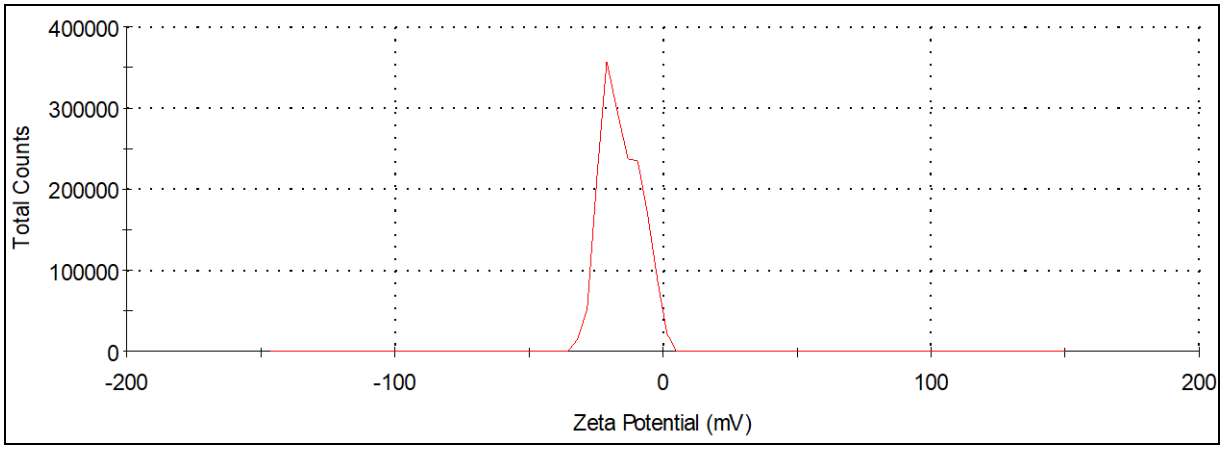


Fig 6: EDAX Spectrum of AuNPs



MD= *M. dactyloides* (Gold Nano particles) Z-Average (d.nm): 266.4

Fig 7: DLS-Size distribution of AuNPs



MD= *M. dactyloides* (Gold Nano particles) Zeta Potential (mV): -15.5

Fig 8: DLS-Zeta Potential of AuNPs

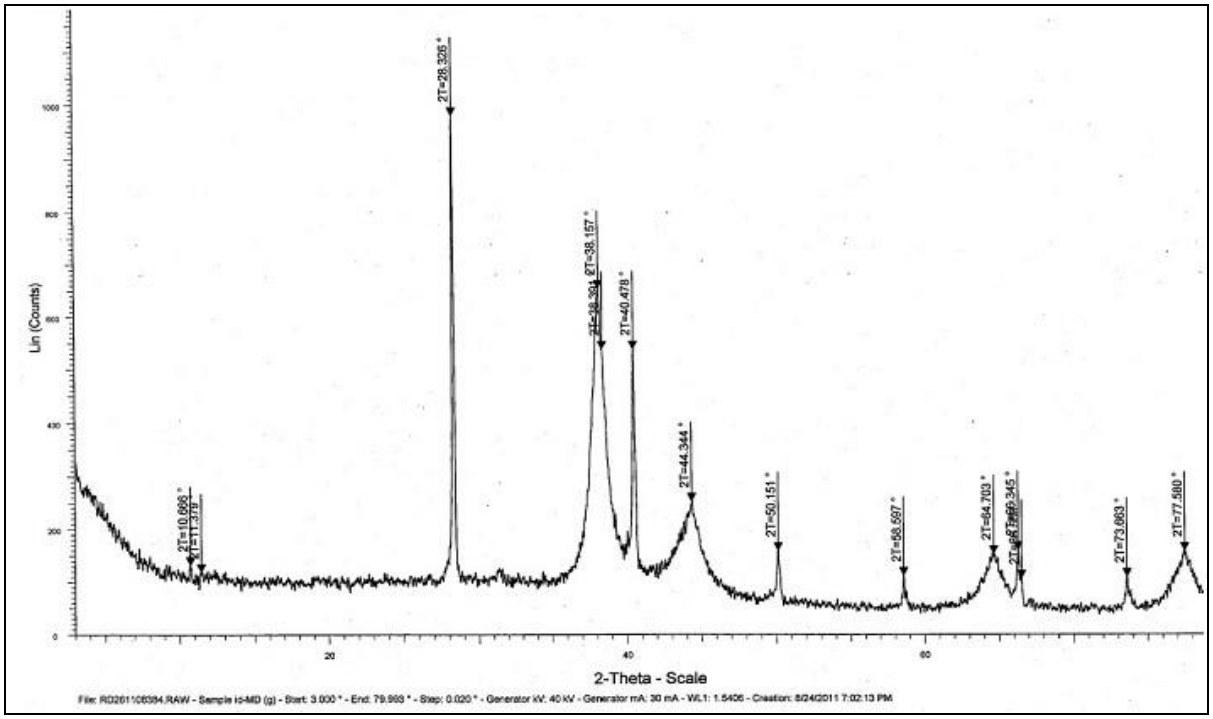


Fig 9: XRD charaterization of AuNPs

Conclusion

In our display investigation, we looked into the mix and characterization of gold nanoparticles, as well as their use in natural frameworks. Gold has a startling range of physical, chemical, and natural properties at the nanoscale. In the antibacterial and anticancer research, a feasible green blend of nanoparticles will have a greater impact and application. We discovered that the Myristica dactyloides will be promising modern pharmaceuticals in the near future as a result of this research.

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