

Isolation, Characterization and Antibacterial Activity of Gold Nanoparticles of *Frangipani* Flower

S.M Gopinath¹, Ismail shareef M², Ashwinipatil G.M*

Professor & HOD, Department of Biotechnology, Acharya Institute of Technology, Bangalore, Karnataka, India¹

Assistant Professor Department of Biotechnology, Acharya Institute of Technology, Bangalore, Karnataka, India²

Research Scholar, Department of Biotechnology, Acharya Institute of Technology, Bangalore, Karnataka, India*

ABSTRACT: The study conducted to assess the characterization and antibacterial activity of gold nanoparticles of *Frangipani* flower. The flower extract used to check antibacterial activity against *Escherichia coli*, *Klebsiella*, *Serratia*, *Staph.epidermis*, *citrobacter* and *Proteus vulgaris* were investigated. Gold nanoparticles using flower extract of *Frangipani* as reducing agent. The complete reduction of gold chloride was observed after 48 h of reaction. The characteristic color changes from pale yellow to dark brown during the formation of gold nanoparticles in the reaction due to their specific properties "Surface Plasmon Resonance" was observed. The flower extracts acts as reducing as well as encapsulate agent for the gold nanoparticles. The UV-Vis spectrophotometer, SEM, TEM and XRD was used to characterize the gold nanoparticles. The UV-visible spectrophotometer indicates a strong Plasmon resonance which is located at 400 nm. The TEM, SEM images show that obtained samples have spherical morphology.

KEYWORDS: *Frangipani*, Gold nanoparticles, antibacterial activity.

I. INTRODUCTION

Nano science and nanotechnology are the study and application of extremely small things and can be used across all the scientific and engineering fields. I.e. about 1 to 100 nanometers. The systems being designed and produced at an incredibly small scale of molecules I.e. nanoscale refer to nanotechnology. Coarse particles range between 10,000 and 2,500 nanometers whereas the fine particles range between 2,500 and 100 nanometers. Ultrafine particles or nanoparticles are sized between 1 and 100 nanometers. The field of science has known for its place in the production of nanomaterials, which are regarded as "First generation" products that includes nanoparticles. Nanoparticles are engineered structures with at least one dimension of 100 nanometers or less. These novel materials are increasingly used for commercial products, including developing new designs as biomedical applications. Using the environmentally benign materials like flower and plant extract, bacteria, fungi for the synthesis of gold nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the protocol.

However, the literature reveals that "inorganic nanomaterials" can be used as potential antimicrobial agents. Now a trend in exploring a novel nanomaterial for antibacterial activity is a burning issue. The experimental results high lightened gold nanoparticles are the promising antibacterial agents can act as a broad spectrum antibacterial agent both extracellular and also intracellular. The mode of action as an antibacterial agent is that the gold ions are bound to the active groups of bacterial cell resulting in the precipitation and inactivation process.

The recent advances in Nano research stated by BSI ("British Standards Institution") nanomaterials are the particles with many dimensions in nanoscale [1]. Usually a novel properties like "optical, magnetic and electrical appear below 100 nanometer size and in case of one dimension in the scale of 1-1000 nm referred as mesoscale nanoparticles. In the

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drug delivery generally, organic polymers play a vital role [2,3]. As per the quantum mechanics stated that in mesoscale atomic or molecular properties are different from the other nanoparticles.

Biosynthesis of nanoparticles using flower and plant extracts are the favorite method of green, the ecofriendly production of nanoparticles exploited to a vast extent because the plants are distributed widely, easily available, safe to handle and with a range of metabolites. The flower material used for the biosynthesis of gold nanoparticles is *Frangipani* flowers and *Ixora coccinea*. Gold and silver metal nanoparticles have been recognized to be important in the fields of science and engineering. These particles are being processed for various purposes because of their remarkable properties such as conductivity, bio-compatibility, optical, photo thermal, magnetic, catalytic and also antimicrobial activity. Other physiochemical factors which are also important are shape, morphology, charge, reactivity and chemical surroundings. Various approaches like chemical reduction for the synthesis of metal nanoparticles has been reported. UV photolysis, gamma irradiation, metal vapor deposition, laser ablation and sputtering among these chemical reductions have been most extensively investigated.

Synthesis, characterization to know the vital properties of nanomaterials and application in the field of medicine and drug delivery system have become thrust areas of research. Nanoparticles having one or more dimensions of the order of 100nm or less have attracted considerable attraction due to their unusual properties and various applications, over their bulk counterparts. Currently, a large number of methods are available to synthesize different types of nanoparticles. Though physical and chemical methods are more popular for nanoparticle synthesis, toxic compounds usage limits their applications. The development of eco-friendly methods of biogenetic production are of more interest due to the simplicity of the procedures and versatility. Due to their amenability to biological functionalization, the biological nanoparticles are finding important applications in the field of medicine. On reduction of gold ions present in aqueous solution of the gold complex in *Frangipani* flower extract and *Ixora coccinea* flower extract can be demonstrated with the change in color which is due to formation of nanoparticles.

II. REVIEW OF LITERATURE

In Ayurveda gold based substances were used to treat many diseases. With the recent advances gold ions are used as potential antibacterial agents and emerging out as a vital molecule against major bacterial pathogens. Microbial biomass can retain relatively high quantities of metal by absorption (passive mode) or by bioaccumulation (activity of viable cells). It has been exposed that several types of inactivated biomass and living organisms have the ability to remove high concentrations of Au^{3+} from solution by converting it to Au^0 [1] and summarizes the advances of Nanoscience and exceptional size dependent properties and usage, commercialization in the field of human welfare.

In the cancer therapy and in molecular diagnostics Padma *et al.*, 2010 discussed the importance of a gold nanoparticle derivative with tool modified oligonucleotides and explained how the specific nucleic acid targets have been explored to more conventional techniques. The gold nanoprobe methods quantifies the unamplified human RNS and detect oncogene transcript and these probes help in differentiating gene expressions of total RNA and minimize RNA degradation [4].

Thirumurugan *et al.*, 2010 developed an ecofriendly process for the synthesis of nanoparticles by leaf extract of *Azadirachta indica* and confirmed the presence of nanoparticles by UV-Vis spectrophotometer and hence, green synthesis of nanoparticles started [5]. Balaprasadankamwar 2010 reported low cost synthesis and non toxic ecofriendly gold nanoparticles in the aqueous medium using *Terminalia catappa* leaf extract [3]. Padma S Vankar *et al.*, 2010 reported rapid and convenient method by the reduction reactions synthesized gold nanoparticles by gold chloride from *Mirabilis Jalapa* flowers and characterized the gold nanomaterials by "TEM, XRD, EDAX and AFM" [4]. Nagajyothiet *et al.*, reported the synthesis of highly dispersed gold nanoparticles (AuNPs) using Dried flower extract of *Carthamus tinctorius* as the reducing agent. After exposing the gold ions to flower extract, rapid reduction of gold ions is observed leading to the formation of AuNPs in solution. UV-VIS spectrum of the aqueous medium containing AuNPs obtained after 25 min (at 40 °C) and after 10 hrs (at room temperature) of the reaction, showed absorption peaks at around 414 and 410 nm respectively [23]. Dried flower extracts of *Achillea wilhelmsii* was used in the synthesis

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of gold nanoparticles by rapid reduction and confirmed by the absorption peak at 580 nm by UV-Vis spectrophotometer was proved by JavadKarimiet al [13].

III. MATERIALS AND METHODS

PREPARATION OF FLORAL EXTRACT: Fresh flowers were collected, washed thoroughly thrice with distilled water. It was finely chopped and 10 grams of the chopped petals were boiled in 100ml of distilled water. It was boiled for 15min by shaking at regular intervals. The conical flasks were kept in a rotatory shaker until it was cooled. The contents were then filtered by using whatman filter paper no.1.

RAPID METHOD OF BIOSYNTHESIS OF GOLD NANOPARTICLES USING FLOWER EXTRACTS: Flower extracts were treated with 1Mm HAuCl₄ and incubated in dark for 03 Hours. After incubation, the color changes from pale yellow to dark one and cherry red to pale brown of a *Frangipani* flower. The color changes indicate the presence of gold nanoparticles thus the solution obtained was purified by repeated centrifugation at 15,000 RPM for 20 min. The samples obtained after incubation were centrifuged at 15000rpm for 15min. The nanoparticles obtained were dried by keeping it in the hot air oven at 6000C for 1 hour. The obtained nanoparticles were weighed and used for further analysis.

PURIFICATION OF GOLD NANOPARTICLES: The samples obtained after incubation were centrifuged at 15000rpm for 15min. The nanoparticles obtained were dried by keeping it in the hot air oven at 6000C for 1 hour. The obtained nanoparticles were weighed and used for further analysis.

Nutrient agar was prepared and steam sterilized. The solid agar plates were inoculated with the organisms (*Escherichia coli*, *Proteus vulgaris*, *Serratia* and *citrobacter*). The plates were inoculated by spread plate method using 90µl of the inoculum. These plates were later used for antibacterial studies.

IV. RESULTS

SYNTHESIS OF GOLD NANOPARTICLES: The incubated samples containing the mixture of HAuCl₄ and floral extract were observed for the presence of Nanoparticles (NP). The presence of NP was observed by the change in color of the solution from Yellow and red to dark brown and cherry red. The change in color varied with variable ratio of the mixture (1:5, 1:25, 1:50, 1:75), lowers the ratio, and lighter is the color.

PURIFICATION OF GOLD NANOPARTICLES BY CENTRIFUGATION: After consecutive centrifugation for three times, the concentrated gold nanoparticle solution was obtained, this was dried under the table lamp of 100W power and then was subjected to desiccation. The dried sample was used for SEM, XRD, TEM and antibacterial activity.

ANTIBACTERIAL ACTIVITY: Antibacterial study conducted on the gold nanoparticles obtained from the floral extract *Frangipani* flower found that the gold nanoparticles had better efficacy against most of the microorganisms shown in the fig designated as plate1, plate2, plate3, plate4, plate5 and plate6 below.

UV-VIS SPECTROPHOTOMETER: After three hours incubating the sample the colored sample was subjected to UV-vis spectrophotometry. In the above process the maximum absorbance was determined with respect to varying wavelength within the range of 400 to 800nm by using the *Frangipani* flower extract, *Frangipani* flower supernatant and *Frangipani* flower solubilize in deionized water shown in table1, table2, table3 and graph1, graph2, graph3 respectively. the list of data is as showed below.

SEM, TEM and XRD: With the help of these micrographs, the average particle size of gold nanoparticles is around 40-60 nm and it are in a spherical shape. TEM analysis helps us to determine the size of the gold nanoparticles shows 100nm the SEM, TEM and XRD shown in the fig1, fig2 and fig3 respectively.

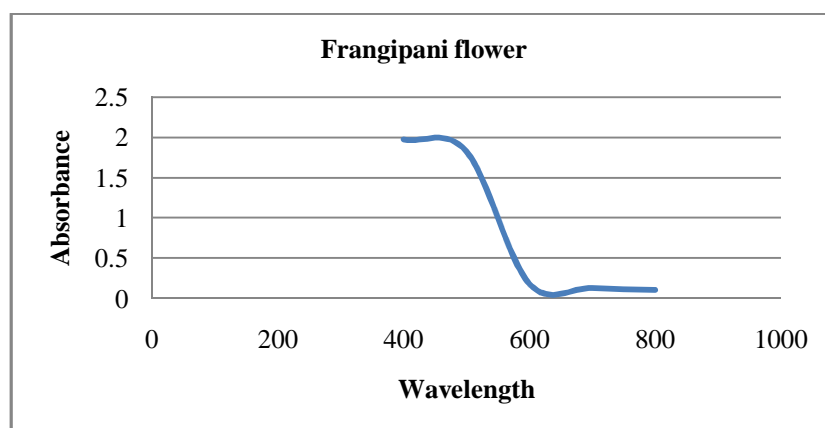
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| Wavelength | Absorbance |
|------------|------------|
| 400 | 1.976 |
| 500 | 1.827 |
| 600 | 0.181 |
| 700 | 0.13 |
| 800 | 0.105 |

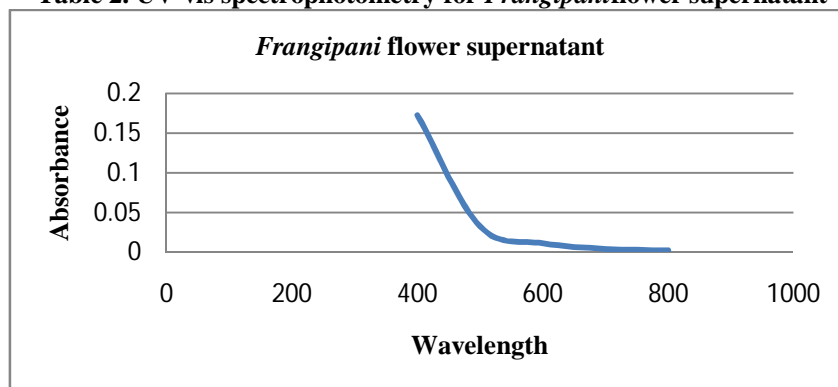
Table.1. UV-vis spectrophotometry for *Frangipaniflower* extract



Graph1. The maximum absorbance obtained for *Frangipani* flower extract was 1.976 at the wavelength of 400 nm.

| Wavelength | Absorbance |
|------------|------------|
| 400 | 0.173 |
| 500 | 0.032 |
| 600 | 0.011 |
| 700 | 0.004 |
| 800 | 0.002 |

Table 2. UV-vis spectrophotometry for *Frangipaniflower* supernatant



Graph2. The maximum absorbance obtained for *Frangipani* flower supernatant was 0.173 at the wavelength of 400 nm

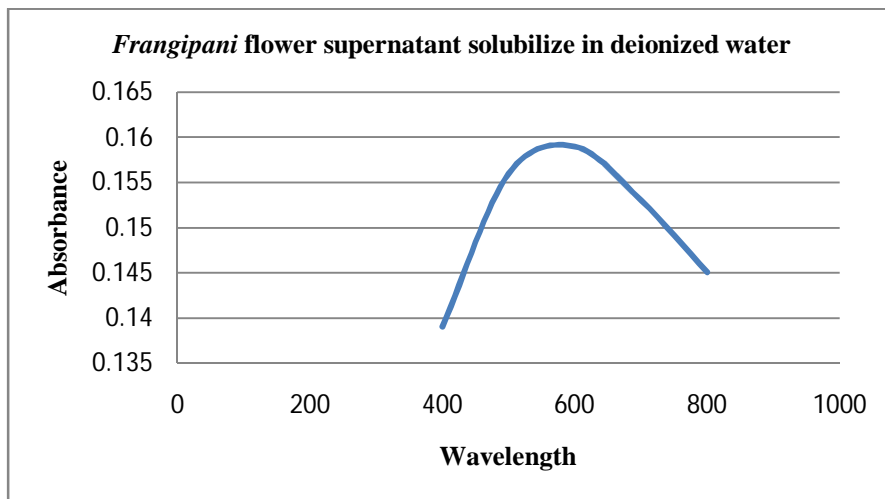
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| Wavelength | Absorbance |
|------------|------------|
| 400 | 0.139 |
| 500 | 0.156 |
| 600 | 0.159 |
| 700 | 0.153 |
| 800 | 0.145 |

Table 3. UV-vis spectrophotometry for *Frangipani* flower supernatant solubilize in deionized water



Graph.3. the maximum absorbance obtained for *Frangipani* flower supernatant solubilize in deionized water was 0.159 at the wavelength of 600 nm.



Plate.1. *Citro bacter* Plate.2. *Klebsiella* Plate.3. *Serratia*

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Plate.4. *E. coli*

Plate.5. *Proteus vulgaris*

Plate.6. *Staph. epidermis*

Antibacterial activity plates

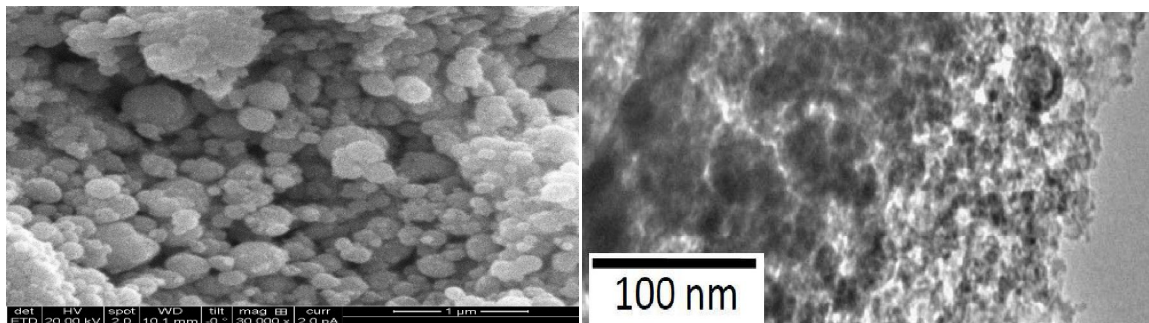


Fig.1. SEM photo of *Frangipani*

Fig.2. TEM photo of *Frangipani*

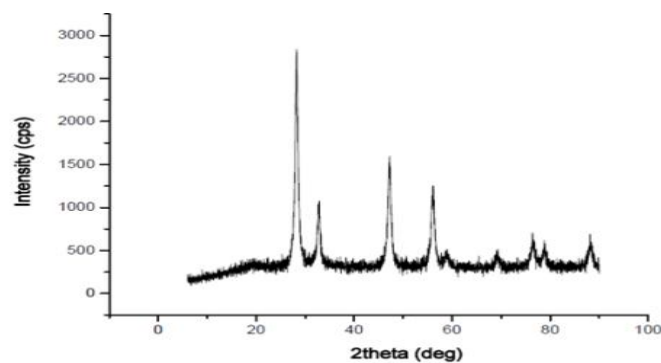


Fig.3. XRD photo of *Frangipani*

V. DISCUSSION

SYNTHESIS OF GOLD NANOPARTICLES

The complex gold particles present in the floral extracts get converted to gold nanoparticles; after undergoing reduction reaction with $AuCl_4$.

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PURIFICATION OF GOLD NANOPARTICLES BY CENTRIFUGATION

The Gold nanoparticles that were obtained was washed with deionized water to remove excess of AuCl₄ and was washed with 70% ethyl alcohol to remove organic and phenolic compounds from the mixture.

UV-VIS SPECTROPHOTOMETER

From the UV-vis spectrophotometry, three types of methodology were used to characterize the flower extract by taking the OD at different wavelengths of flower extract, supernatant and supernatant with deionized water. The maximum absorbance obtained for *Frangipani* flower extract was 1.976 at the wavelength of 400 nm. Whereas, the maximum absorbance obtained for *Frangipani* flower supernatant was 0.173 at 400nm. The final absorbance was done with the flower supernatant with deionized water of *Frangipani* was found to be 0.159 at 600 nm .

Though the maximum absorbance obtained varied over a range of 0.490 to 1.976, but they were obtained within the common range of wavelength of 400 to 500 nm confirming the presence of Gold nanoparticles.

ANTIBACTERIAL ACTIVITY

Antibacterial study conducted on the gold nanoparticles obtained from the floral extract *Frangipani* flower found that the gold nanoparticles had better efficacy against most of the microorganisms. However, it was found to be the most effective in *Frangipani* flower extract at the most efficacy on *staph epidermis* and *E. coli* showing a 9cm and 6 cm respectively. Finally, it was observed that amongst all types of gold nanoparticles obtained from different floral extracts the maximum efficacy was against the microorganism *E. coli* and *Staph epidermis*.

The antibacterial activity displayed by the small quantity of gold nanoparticles used against microorganisms and can be effective in the treatment of various diseases.

SEM, TEM and XRD

With the help of these micrographs, the average particle size of gold nanoparticles is around 40-60 nm and it are in a spherical shape. TEM analysis helps us to determine the size of the gold nanoparticles shows 100nm this is due to the fact that during transmission electron microscopy the electrons penetrate through the particle and beam of electron are studied. SEM analysis helps us to determine the morphology of the particle since the electrons from the surface are reflected and the beam of these reflected electrons was scanned. The size value is in accordance with the theoretical value of the size of the gold nanoparticles based on JCPDS data as per XRD analysis.

VI. CONCLUSION

By simple and effective synthesis of gold nanoparticles was successfully produced by *Frangipani* flower extract. The flower extract for nanoparticle synthesis can be advantageous over other biological methods. Following the addition of the gold chloride solution, gold nanoparticles began to form within 15 minutes and the reaction neared completion at 2h, as shown by the UV -Vis spectrophotometry. Using reducing agents like gold chloride can be used in the synthesis of gold nanoparticles earlier in ethanomedicine used to treat neurological disorders. The synthesized gold nanoparticles show potential bactericidal activity against 04 major bacterial pathogens. It was found that the increasing in the concentration increases the rate of reduction and reduces the particle size as well as their agglomeration. The reduction of gold ions to gold nanoparticles was found to be optimized at a ratio of 1:20 of flower extract. The synthesized particles ranged in size from 10 -25nm and were spherical in shape, as shown by the SEM, TEM and XRD analysis.

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