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Environmental Benign synthesis of gold Nanoparticles from the flower extracts of P*lumeria Alba* Linn. (Frangipani) and Evaluation of their Biological activities

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Abstract

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. Here we report extracellular biosynthesis of gold nanoparticles using flower extracts of Plumeria alba as reducing agent. The complete reduction of auric chloride was observed after 48 h of reaction at 30 ºC. 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 encapsulating agent for the gold nanoparticles. The UV-Vis spectroscopy and transmission electron microscopy (TEM) was used to characterize the obtained gold nanoparticles. The UV-visible spectra indicate a strong Plasmon resonance that is located at ~550 nm. The TEM images show that obtained samples have spherical morphology with two different size particles smaller particles with 20-30 nm size and bigger one with 80-150 nm particles. The antimicrobial activities of these gold nanoparticles against different microorganisms were also studied.

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INTRODUCTION

Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nano size. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles [1].

A number of approaches are available for the synthesis of gold and silver nanoparticles for example, reduction in solutions [2], chemical and photochemical reactions in reverse micelles [3], thermal decomposition of silver compounds [4], radiation assisted [5], electrochemical [6], sono-chemical [7], microwave assisted process [8] and recently via green chemistry route [9][10][11]. The use of environmentally benign materials like plant leaf extract [12] , bacteria [13], fungi [14] and enzymes [15] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications of these nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

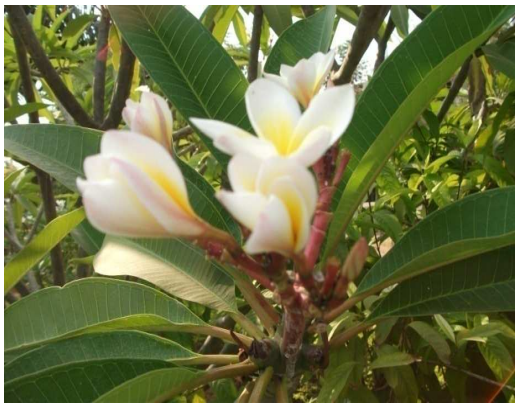
We herein report the synthesis of gold nanoparticles by the reduction of aqueous AuCl4- with the flower extract of *Plumeria alba* Linn. (Frangipani flower). The *Plumeria alba* Linn comes under the Kingdom- Plantae, Family – Apocynaceae, Genus- *Plumeria*, Species- *P. alba*.

This large evergreen shrub has narrow elongated leaves, large and strongly perfumed white flowers with a yellow center. Native from Central America and the Caribbean, it is now common and naturalized in southern and southeastern Asia. *Plumeria alba* Linn (Apocynaceae) is used in the treatment of ulcers, herpes, scabies and seeds possess haemostatic properties. The bark is bruised as plaster over hard tumours [16-17], whereas, the latter taxon finds use as purgative, cardiotonic, diuretic and hypotensive [18-19]. Methanolic extract showed antimicrobial activity against Bacillus anthracis, Pseudomonas aeruginosa[20].

The essential oils from the flowers are used for perfumery and aromatherapy purposes. The decoction of the bark and roots of *P. rubra* is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation [21]. The fruit is reported to be eaten in West Indies. In India, however, it has been used as an abortifacient [22]. The flowers are aromatic and bechic and widely used in pectoral syrups.The flowers decoction of *P.rubra* was reported to use in Mexico for control of diabetes mellitus. The Leaves of *P.rubra* are used in ulcers, leprosy, inflammations and rubefacient [23].

Leaves are useful in inflammation, rheumatism, antibacterial, bronchitis, cholera, cold and cough, Antipyretic, antifungal, stimulant etc. [24]. Looking to the scope of herbal drug and increasing demand, especially in disease of liver, cancer, diabetes, hypertension, renal disease, inflammation, infectious and skin diseases. The selection of the plant *Plumeria rubra* Linn is made on the basis of its easy of availability, therapeutic value and degree of research work which is not done [25].

Figure 1. Frangipani Flower



MATERIALS AND METHODS:

Flower extraction

The fresh flowers (20g) of Frangipani flower samples were collected from our college campus itself and authenticated by the department of Applied Botany, University of Mysore. Collected fresh flowers were washed, finely cut and soaked in 100ml boiling distilled water for 5-10 min and then it was filtered through Whatman filter paper no.1.

Synthesis of gold nanoparticles:

In a typical experiment gold nanoparticles were synthesized by taking 5ml of flower extract was added into 45ml 0.002M $AuCl_4$ obtained from Loba Chemie Pvt. Ltd. Mumbai and kept in dark for 3-4 hours. Within an hour pale yellow solution was obtained. The gold nanoparticles solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant is discarded and the pellet is dissolved in deionized water. The bioreduction of Au^{3+} in aqueous solution was monitored by periodic sampling of aliquots of the suspension. The synthesized nanoparticles were screened for its antibacterial and antifungal activity by disc method.

Characterization

The gold nanoparticles characterized by Elico SL 164 double beam UV-Visible Soectrophotometer [26]. The morphology of the samples was studied by high-resolution transmission electron microscopy (HRTEM; JEOL JEM-2010F).

Antifungal and antibacterial activity

Aspergillus niger, Aspergillus flavus, E.coli and *Streptobacillus sp.* collected from authenticated stock culture of our college itself.

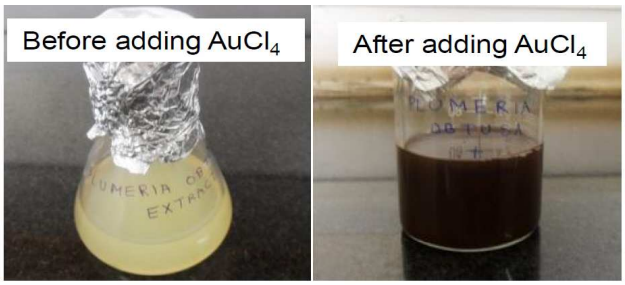
Culturing of Potato Dextrose Media

2.4 g of potato dextrose broth and 2 g of agar are dissolved in 100 ml of distilled water. The contents are subjected to autoclaving at 121 ^{o}C for 20 min at 15lbs pressure. Potato dextrose agar media plate is prepared by pouring the nutrient agar media into the petriplates. The microbial suspension of *Aspergillus niger* and *Aspergillus flavus* was spread over the media. The standard antibiotic disc was also placed in one side of the petriplates which is the control and the pretreated antibiotic discs with the synthesized nanoparticles in another side. The inoculated petriplates is covered and it is kept for incubation at room temperature.

RESULTS AND DISCUSSION

Gold nanoparticles were synthesized from Hydrogen tetra chloroaurate solution containing Au^{+} ions by treating with the *Plumeria alba* flower extracts. The color of the solution changed to deep brownish color within 30 min of reaction with the Au^{+} ions. The appearance of the deep brownish color indicated formation of gold nanoparticles (Fig 2). They turned brown and the intensity of color was increased with the time of incubation.

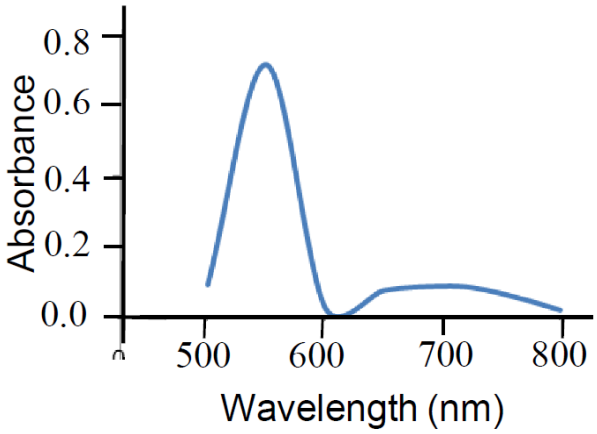
Figure 2. Test for Frangipani flower to show the synthesis of gold nanoparticles



The formation of gold nanoparticles was confirmed by color changes followed by UV- Visible spectrophotometer analysis. The UV- Visible spectrophotometer has proved to be very useful technique for the analysis of some metal nanoparticles. The UV- visible spectra (shown in Graph 1) indicate a strong Plasmon resonance that is located at ~550 nm. Presence of this strong broad

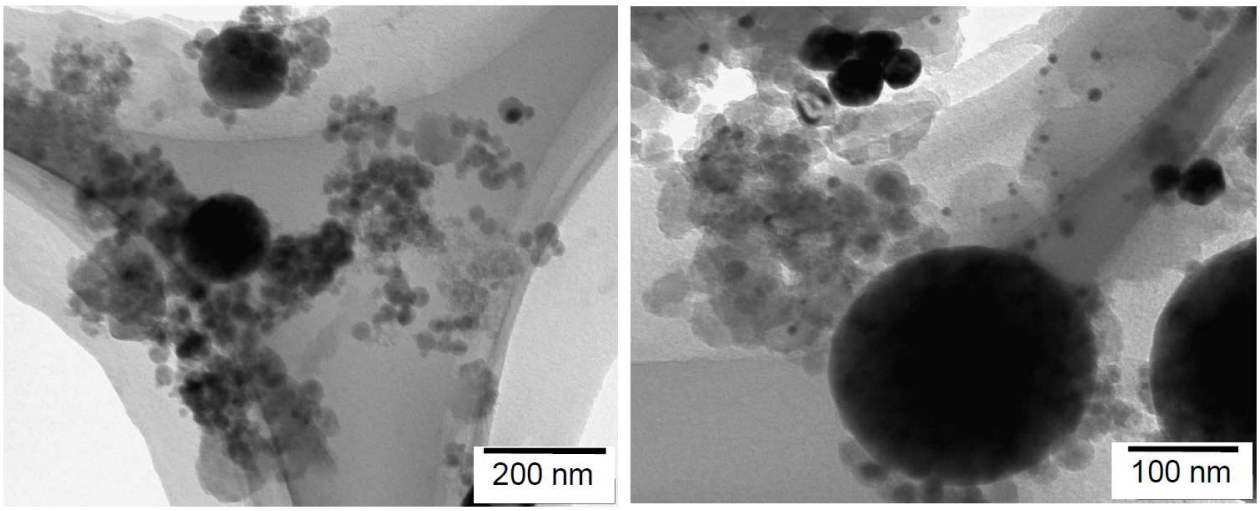
plasmon peak has been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm [27].

Graph 1. UV-Visible spectrum of gold nanoparticles synthesized using Frangipani flower extract.



The microstructures and size of the biosynthesized gold nanoparticles were studied by Transmission Electron Microscopy (TEM) analysis. The typical TEM images of the gold nanoparticles synthesized by Frangipani flower extract as reducing agent is shown in Fig. 3. The micrograph shows formation of spherical nanoparticles with different size. The smaller particles are about 20-30 nm in size whereas bigger sphere type particles are about 80-150 nm in size. Interestingly we observed that the smaller particles were assembled around the bigger particles as shown in Fig 3, which indicates that the flower extract also acts as some capping agent.

Figure 3. TEM images of gold nanoparticles synthesized from Frangipani flower spherical morphology.



The gold nanoparticles obtained from flowers of *Plumeria alba* were tested against set of microorganisms in order to estimate their antimicrobial potentials. Anti bacterial study indicates that antibiotic with gold nanoparticles extracted from Frangipani flower (A+F) exhibit more zone of inhibition compared to standard antibiotics (A) used (Table 1 and Graph 2). The zone of inhibition against *E.coli* is more when compared with the zone of inhibition exhibited on *Streptobacillus sp.* for gold nanoparticles with antibiotics like Imipenem, Norfloxacin and Vancomycin (Fig. 4). Imipenem and Vancomycin with gold nanoparticles has less zone of inhibition against *E.coli* and *Streptobacillus sp.* (18mm and 16mm respectively), where as gold nanoparticles with Norfloxacin shows more inhibition against *E.coli* and *Streptobacillus sp.* (24mm and 24mm respectively). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+F) shows more zone of inhibition against *E.coli* and *Streptobacillus sp.*(31mm and 24mm respectively).

Table 1. Antibacterial activity of the gold nanoparticles synthesized from Frangipani flower

Antibiotics	Diameter of zone of inhibition in mm							
	Organisms							
	Aspergillus niger		*Aspergillus flavus*		*E.coli*		*Streptobacillus sp.*	
	A	A+F	A	A+F	A	A+F	A	A+F
Imipenem	-	10	-	-	20	24	16	18
Norfloxacin	-	-	24	30	22	24	23	24
Vancomycin	24	26	25	26	20	31	14	16

Graph 2. Antimicrobial activity of extracted gold nanoparticles from Frangipani flower

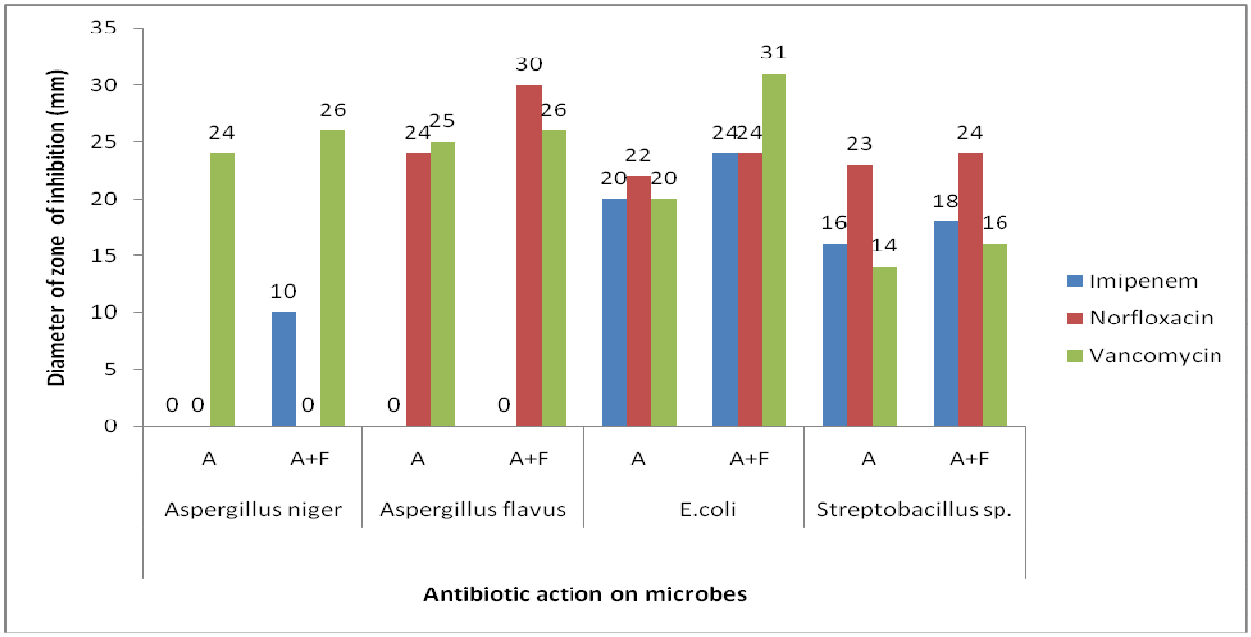
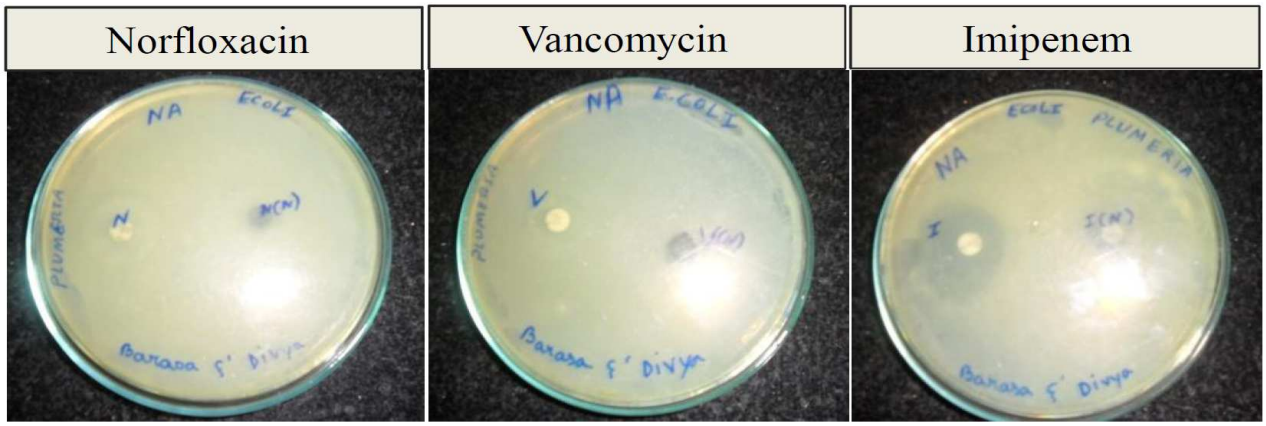
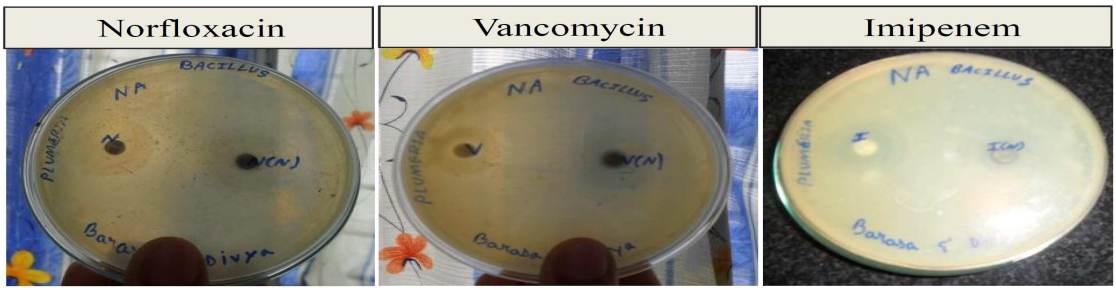


Figure 4. Antimicrobial activity of the gold nanoparticles synthesized from Frangipani against *E.coli*



In antifungal activity, compare to *Aspergillus niger, Aspergillus flavus* shows more zone of inhibition for the antibiotics Norfloxacin and Vancomycin in both antibiotics (A) and antibiotics with Frangipani extract (A+F). Both Norfloxacin and Vancomycin with gold nanoparticles does not show any reaction against *Aspergillus niger* but against *Aspergillus flavus* shows more inhibition (Graph 2) (30mm and 26mm respectively). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+F) shows more zone of inhibition against *Aspergillus flavus*.

Figure 5. Antimicrobial activity of the gold nanoparticles synthesized from Frangipani against *Streptobacillus sp.*



Some authors have reported the preliminary phytochemical screening of flowers of Plumeria Alba, which revealed the presence of Steroid, Flavonoid and alkaloid in *P. Alba* flower extracts [28]. The antitumour activity of the methanolic extract of Plumeria alba leaves (MPA) was evaluated against EAC and DLA using in-vitro cytotoxic and mean survival time [29]. Zahid et al. studied the antimicrobial activity of essential oil from *Plumeria alba* flowers but the results shows 35mm for oil against *E. Coli* but our results shows 31mm against the same species for gold nanoparticles.

Ajay Sing et al. evaluated anti bacterial activity of various extract of powdered leaves of *Plumeria Rubra* [30] against E.Coli and observed the zone of inhibition as 21 mm. Antibacterial activity of *Plumeria alba* (Frangipani) [31] petals methanolic extracts were evaluated against *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus saprophyticus,Enterococcus faecalis* and *Serratia marcescens* by using disk diffusion method extracts (80 %) showed the highest inhibition zone towards *Escherichia coli* (14.3 mm) but in our report flowers shows 31 mm. Ours is the only report for both synthesis of gold nanoparticles as well as antimicrobial activity for flowers of *Plumeria alba*

CONCLUSION

In conclusion, our study can be considered as the first report for the synthesis of gold nanoparticles using extracts of *Plumeria alba* flower samples. Gold nanoparticles were confirmed by color changes and were characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 550 nm for gold nanoparticles. The micrograph shows formation of spherical nanoparticles. The sizes of the nanoparticles were in the range of 10-50 nm, showing a broad size distribution. This technique has proved to be very useful for the analysis of nanoparticles. *Plumeria alba* appears to have significant antimicrobial capacity resembling a broad spectrum antibiotic against the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and are relatively resistant towards synthetic drugs.

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