WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 9, 1410-1422. Research Article ISSN 2277– 7105

COMPARATIVE ANALYSIS OF ISOLATION CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF GOLD NANOPARTICLES OF *FRANGIPANI* AND *IXORA COCCINEA*

[1]S.M.Gopinath, [2]Ismail shareef M, [3]Ashwinipatil G.M*

[1]Professor&HOD, Dept.of Biotechnology, Acharya Institute of Technology, Bangalore.
[2]Assistant professor, Dept.of Biotechnology, Acharya Institute of Technology, Bangalore.
*[3]Research scholar, Dept.of Biotechnology, Acharya Institute of Technology, Bangalore.

Article Received on
26 June 2015,

Revised on 16 July 2015,
Accepted on 10 Aug 2015

***Correspondence for Author**
Ashwinipatil G.M
Research scholar
Dept.of Biotechnology
Acharya Institute of
Technology Bangalore.

ABSTRACT

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 flower material used for the biosynthesis of gold nanoparticles is *Frangipani* flowers and *Ixora coccinea.* Gold nanoparticles have been recognized to be important in the fields of science and engineering and the study investigates the antibacterial activity against different microorganisms.

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

INTRODUCTION

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.

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 gold ions present in aqueous solution of the gold complex in *Frangipani* flower extract and *Ixoracoccinea* flower extract can be demonstrated with the change in color which is due to formation of nanoparticles.

MATERIALS AND METHODS

ISOLATION OF GOLD NANOPARTICLES

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 whattman filter paper no.1.

Preparation of Gold Chloride (Chloroauric Acid)

1mM Gold chloride ($HAucl_4$) was prepared by adding 0.008gms of Gold chloride in 100ml of distilled water.

Incubation

The floral extract and 1mM $HAucl_4$were mixed together in the conical flask and incubated for 3 hours in the dark. Thereafter the absorbance was measured using a UV- vis Spectrophotometer.

Rapid method of biosynthesis of gold nanoparticles using flower extracts

Flower extracts were treated with 1Mm $HAucl_4$ and incubated in dark for o3 Hours. After incubation, the color changes from pale yellow to dark one and cherry red to pale brown of *a Frangipani* flower and *Ixoracoccinea* flowers respectively. 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 collected pellet is dissolved in deionized water the gold nanoparticles was confirmed and characterized by UV-visible spectrophotometer.

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 600^0C for 1 hour. The obtained nanoparticles were weighed and used for further analysis.

Preparation of Nutrient Agar

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

RESULTS AND DISCUSSION

Synthesis of Gold Nanoparticles

The incubated samples containing the mixture of $HAucl_4$ 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 colour varied with variable ratio of the mixture (1:5, 1:25, 1:50, 1:75), lowers the ratio, lighter is the color.

Purification of gold nanoparticles by centrifugation

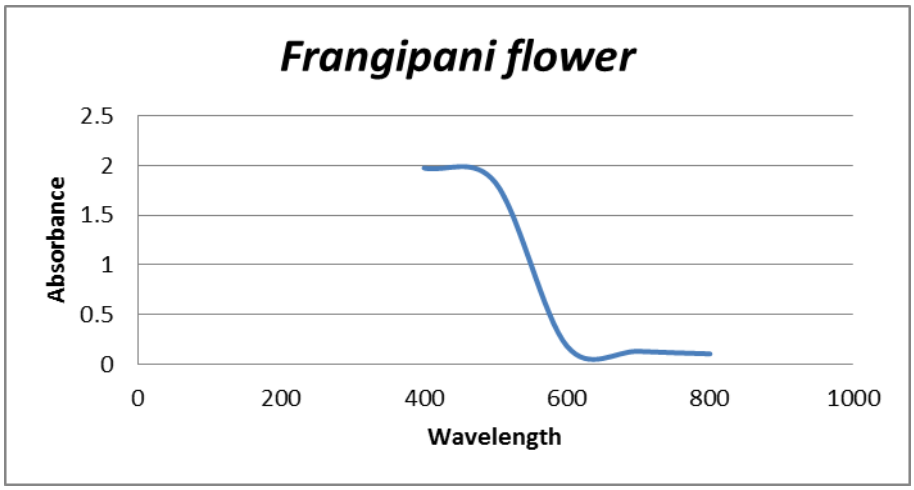
After consecutive centrifugation for three times, the concentrated gold nanoparticle solution was obtained, this was dried under the table lamp of 100W power and then was subjected to desiccation. The dried sample was used for SEM, XRD, TEM and antibacterial activity.

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. The list of data is as follows.

Table.1. UV-vis spectrophotometry for *Frangipani* flower extract

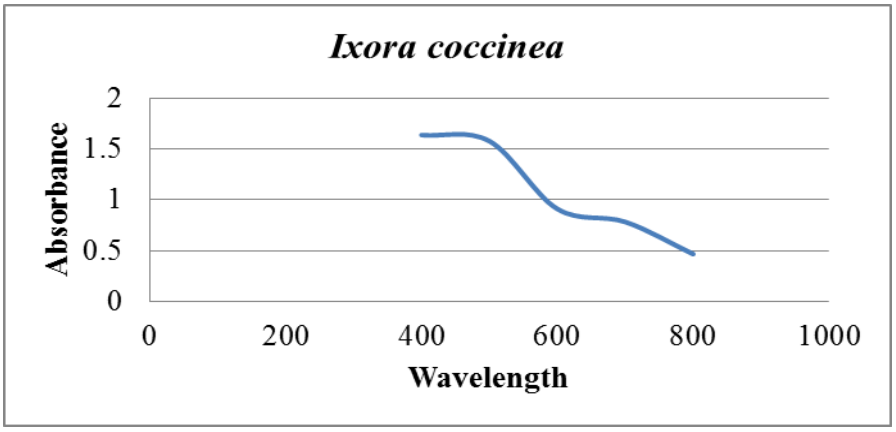
Wavelength	Absorbance
400	1.976
500	1.827
600	0.181
700	0.13
800	0.105



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

Table.2. UV-vis spectrophotometry for *Ixora coccinea* extract

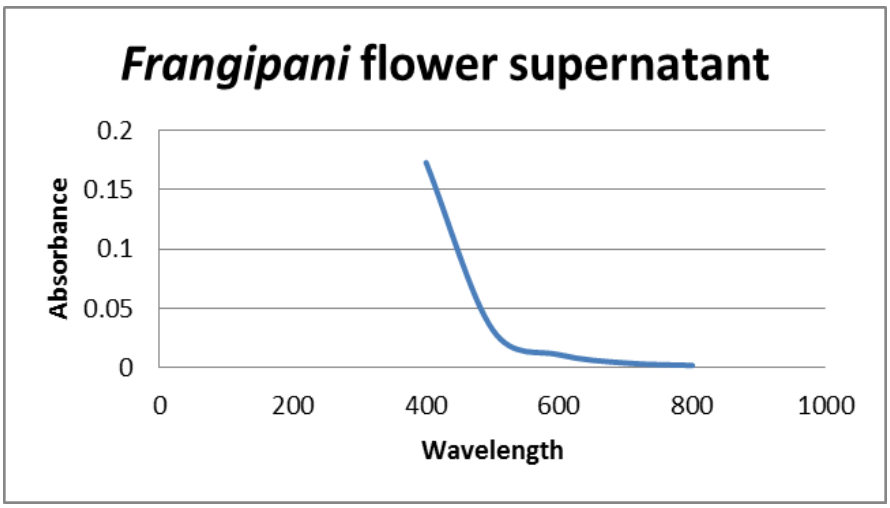
Wavelength	Absorbance
400	1.640
500	1.579
600	0.912
700	0.784
800	0.467



Graph2. The maximum absorbance obtained for *Ixora coccinea* flower extract was 1.640 at the wavelength of 400 nm.

Table 3. UV-vis spectrophotometry for *Frangipani* flower supernatant

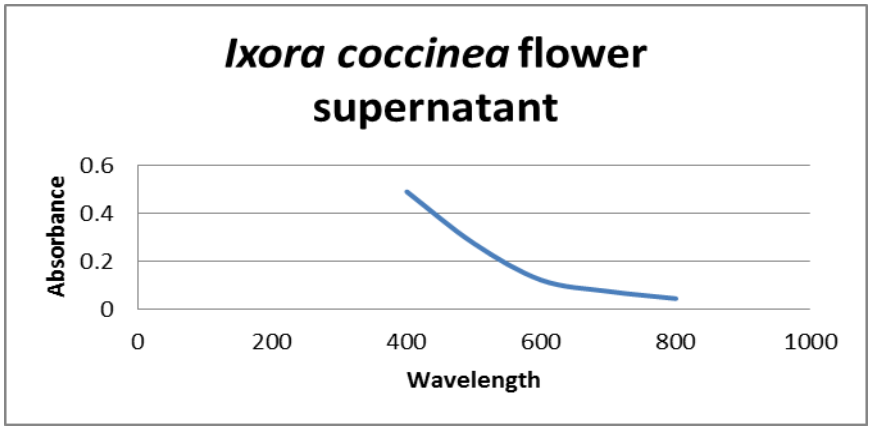
Wavelength	Absorbance
400	0.173
500	0.032
600	0.011
700	0.004
800	0.002



Graph3. The maximum absorbance obtained for *Frangipani* flower supernatant was 0.173 at the wavelength of 400 nm.

Table 4. UV-vis spectrophotometry for *Ixora coccinea* flower supernatant

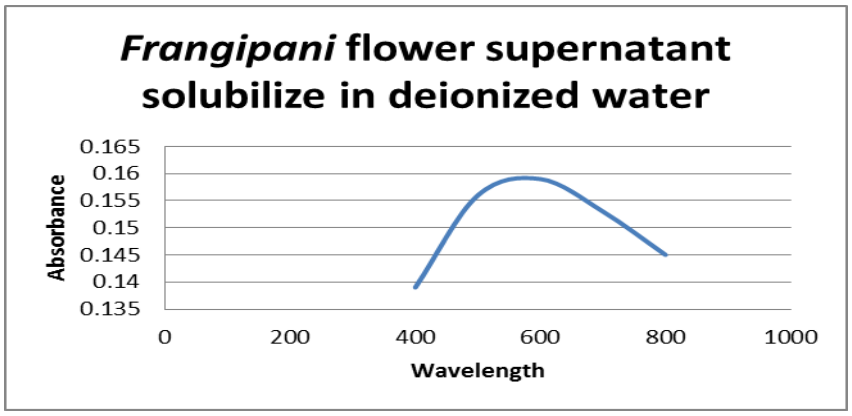
Wavelength	Absorbance
400	0.490
500	0.274
600	0.122
700	0.075
800	0.045



Graph4. The maximum absorbance obtained for *Ixora coccinea* flower supernatant was 0.490 at the wavelength of 400 nm.

Table 5. UV-vis spectrophotometry for *Frangipani*flower supernatant solubilize in deionized water

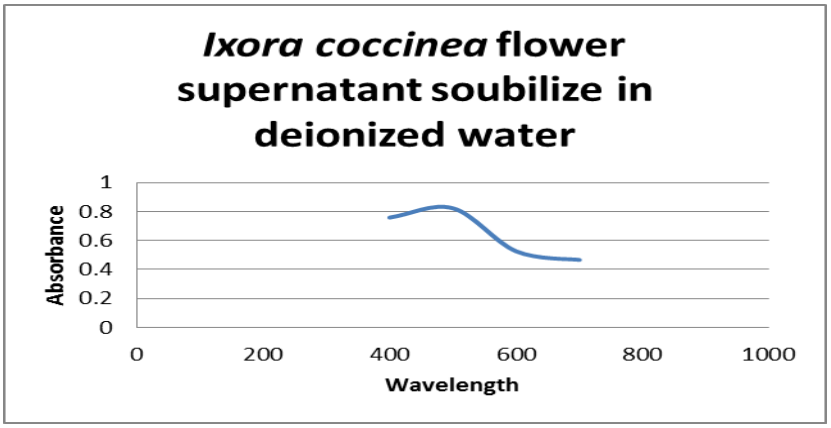
Wavelength	Absorbance
400	0.139
500	0.156
600	0.159
700	0.153
800	0.145



Graph.5. The maximum absorbance obtained for *Frangipani* flower supernatant solubilize in deionized water was 0.159 at the wavelength of 600 nm.

Table 6. UV-vis spectrophotometry for *Ixora coccinea* flower supernatant solubilize in deionized water

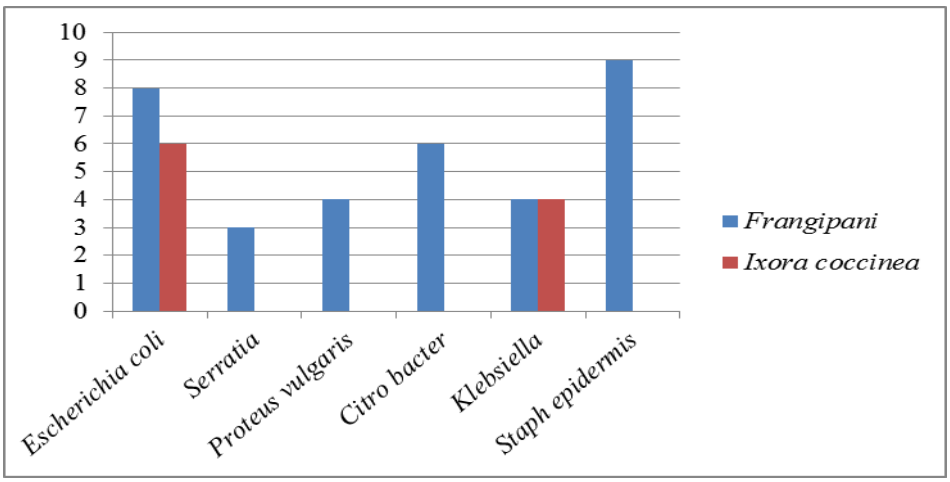
Wavelength	Absorbance
400	0.759
500	0.823
600	0.526
700	0.467
800	0.432



Graph6. The maximum absorbance obtained for *Ixora coccinea* flower supernatant solubilize in de ionized water was 0.823 at the wavelength of 500 nm.

Table.7 Zone of Inhibition of flower extracts against different bacteria

Flower extracts	*Escherichia coli*	*Serratia*	*Proteus vulgaris*	*Citrobacter*	*Klebsiella*	*Staph epidermis*
Frangipani flower	8	3	4	6	4	9
Ixoracoccinea	6	0	0	0	4	0



Graph.7.The maximum zone of inhibition of flower extracts against different bacteria

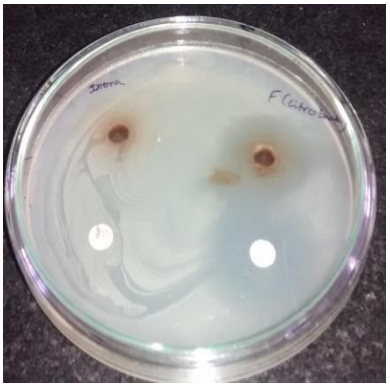


Plate.1 *Citro bacter*

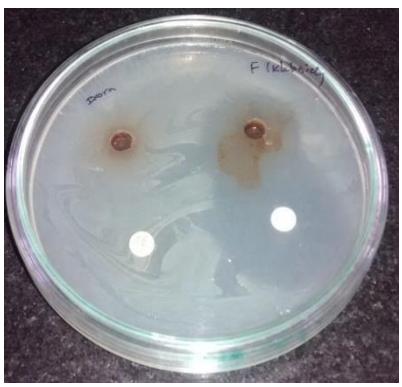


Plate.2 *Klebsiella*

Plate.3. *Serratia*

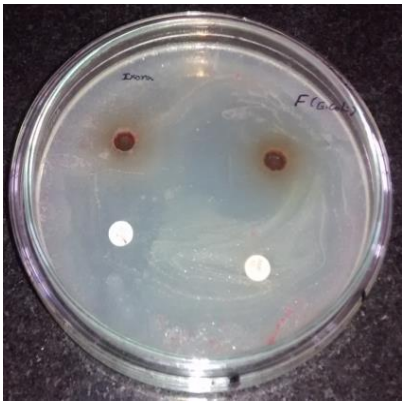


Plate.4 *E.coli*

Plate.5 *Proteus vulgaris*

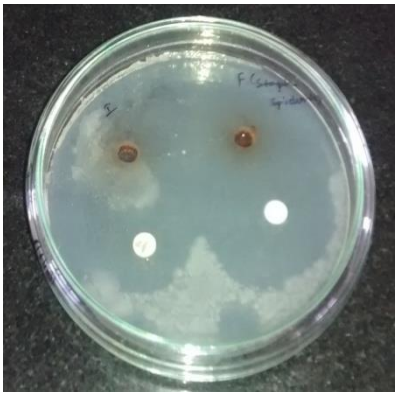


Plate.6. *Staph.epidermis*

Antibacterial activity plate photos

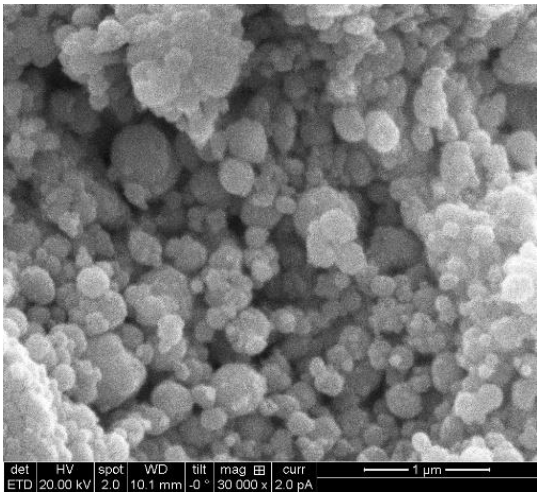


Fig.1.SEM Photograph of *Frangipani*

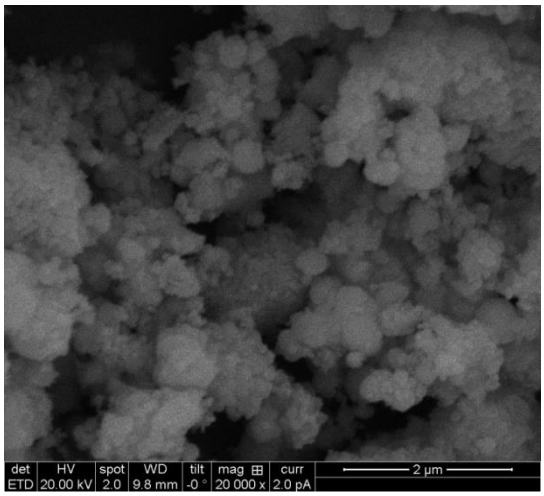


Fig.2. SEM Photograph of *Ixora coccinea*

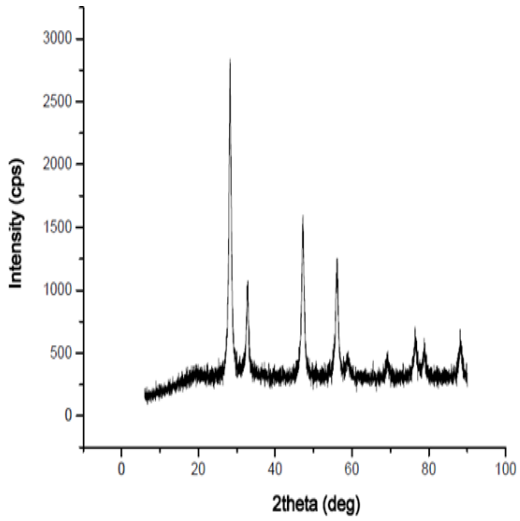


Fig.3. XRD of *Frangipani*

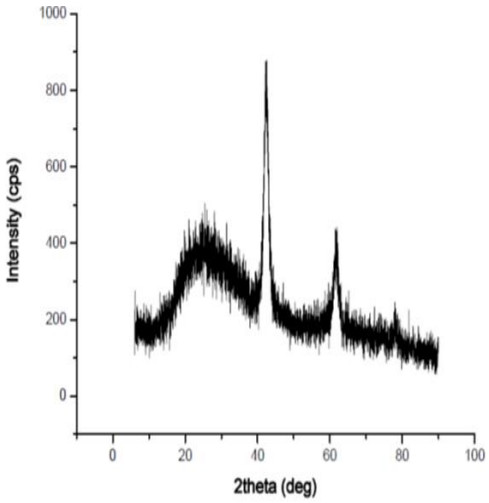


Fig.4. XRD of *Ixora Coccinea*

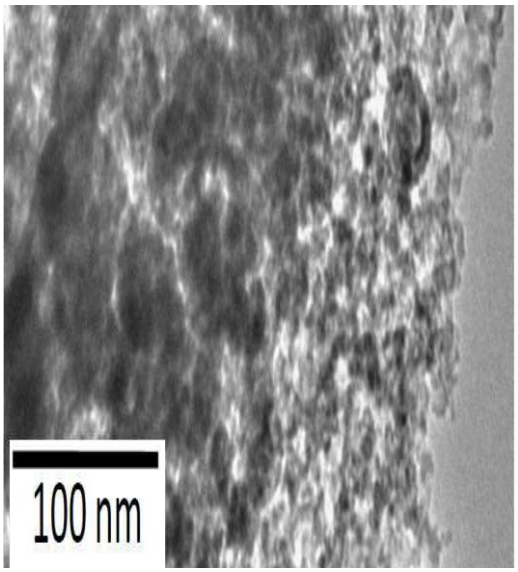


Fig.5.TEM Photograph of *Frangipani*

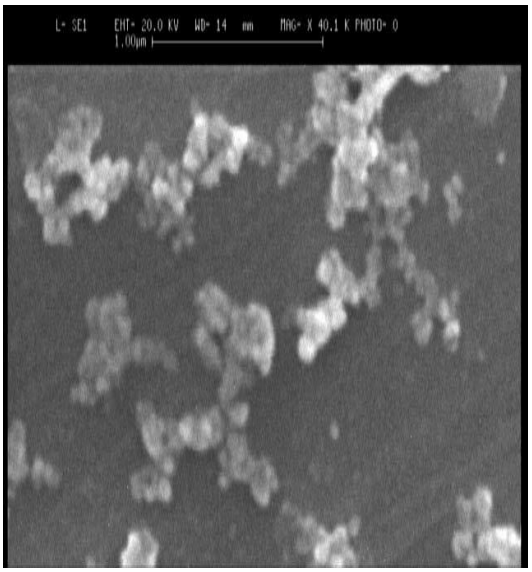


Fig.6.TEM photograph of *Ixora coccinea*

DISCUSSION

Synthesis of Gold Nanoparticles

The complex gold particles present in the floral extracts get converted to gold nanoparticles; after undergoing reduction reaction with $Aucl_4$.

Purification of Gold Nanoparticles by Centrifugation

The Gold nanoparticles that were obtained was washed with deionized water to remove excess of $Aucl_4$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 two different flower extracts by taking the OD at different wavelengths of flower extract, supernatant and supernatant with deionized water.

The maximum absorbance obtained for *Frangipani* flower extract and *Ixora coccinea* flower extract was respectively 1.976 and 1.640 at the wavelength of 400 nm. Whereas, the maximum absorbance obtained for Frangipani flower supernatant and *Ixora coccinea* supernatant was 0.173 and 0.490 at 400 and 600 nm respectively. The final absorbance was done with the two flower supernatant with deionized water of *Frangipani* and *Ixora* was found to be 0.159 and 0.823 at 600 and 500 nm respectively.

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 two floral extracts like *Frangipani* flower and *Ixora coccinea* 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. Similarly for *Ixora coccinea* the zone of inhibition and most effective on *E.coli* and *Klebsiella* of 6cm and 4cm 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.

CONCLUSION

By simple and effective synthesis of gold nanoparticles was successfully produced by *Frangipani* and *Ixora coccinea* flower extracts. The flower extracts 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.

REFERENCES

1. Wenxing Wang , Qifan Chen, Cha Jiang, Dongzhi Yang, Xingmin Liu, Shukun Xu: One-step synthesis of biocompatible gold nanoparticles using gallic acid in the presence of poly-(N-vinyl-2-pyrrolidone). *Colloids and Surfaces A:Physicochem. Eng. Aspects.,* 2007*;* 301: 73–79.
2. Balaprasad Ankamwar: Biosynthesis of Gold Nanoparticles (Green-Gold) Using LeafExtract of Terminalia Catappa. ISSN: 0973-4945; *CodenEcjhao E-Journal of Chemistry., 2010;* 7(4): 1334-1339.
3. Padma S Vankar and DharaBajpai: 2010. Preparation of gold nanoparticles from MirabilisJalapa flowers. *Indian Journal of Biochemistry &Biophysics., 2010;* 47: 157-160.
4. A.Thirumurugan, Jiflin.G.J, Rajagomathi.G,NeethuAnnsTomy, S.Ramachandran, and R.Jaiganesh: 2010. Biotechnological synthesis of gold nanoparticles of Azadirachtaindica leaf Extract.*International Journal of Biological Technology., 2010;* 1(1): 75-77

5. Wang, YixianGao, Liqin Lin, Qingbiao Li, Ling Lin, Mingming Du: 2010. Biosynthesis of GoldNanoparticles by Foliar Broths: Roles of Biocompounds and Other Attributes of the Extracts. *Nanoscale Res Lett.* 5:1351–1359 DOI 10.1007/s11671-010-9652-8.
6. M. Banoeel, N. Mokhtaril, A. AkhavanSepahi, P. JafariFesharaki, H. R. Monsef-Esfahani, Z.Ehsanfar, M. R. Khoshayand and A. R. Shahverdi: 2010. The Green Synthesis Of Gold Nanoparticles Using The Ethanol Extract Of Black Tea And Its Tannin Free Fraction. *IranianJournal of Materials Science & Engineering.* Vol.7, Number 1.
7. Umesh Kumar Parida, Birendra Kumar Bindhani, PadmalochanNayak: Green Synthesis and Characterization of Gold Nanoparticles Using Onion (Allium cepa) Extract. *World Journal of Nano Science and Engineering., 2011;* 1: 93-98.
8. Ratul Kumar Das, NayanmoniGogoi, Utpal Bora: 2011. The Green synthesis of gold nanoparticles using Nyctanthesarbortristis flower extract.*Bioprocess and Biosystems Engineering., 2011;* 34(5): 615-619.
9. Yi Xia Zhang, Jun Zheng, Guo Gao, Yi Fei Kong, Xiao Zhi, Kan Wang, Xue Qing Zhang,Da Xiang Cui: 2011. Biosynthesis of gold nanoparticles using chloroplasts. *Dove Press Journal: International Journal Of Nanomedicine.*
10. SontaraKonwar Boruah, Prabin Kumar Boruah,PradyutSarma, ChitraniMedhi and Okhil Kumar Medhi. Green Synthesis Of Gold Nanoparticles Using Camellia Sinensis And Kinetics Of The Reaction. Department of Chemistry, Gauhati University.
11. Nagaraj B, Krishnamurthy Nb, Dinesh R, Liny P,DivyaTk: 2011. Biosynthesis Of Gold Nanoparticles Of IxoraCoccinea Flower Extract & Their Antimicrobial Activities. *InternationalJournal of Pharma and Bio Sciences.* Vol 2/Issue4.
12. JavadKarimiAndeani, HojjatollahKazemi, SasanMohsenzadeh, AfsanehSafavi: 2011.
13. Biosynthesis Of Gold Nanoparticles Using Dried Flowers Extract Of AchilleaWilhelmsii Plant Digest Journal of Nanomaterials and Biostructures., 2011; 6(3): 911 - 917.
14. Sunil Pandey, Goldie Oza, AshmiMewada and Madhuri Sharon: Green Synthesis of Highly Stable Gold Nanoparticles using Momordicacharantia as Nano fabricator. Archives of Applied Science Research., 2012; 4(2): 1135-1141.
15. Radhika Rajasree SR, Suman TY: 2012.Extracellular biosynthesis of gold nanoparticles using a gram negative bacterium Pseudomonas fluorescens. Asian Pacific Journal of Tropical Disease. S: 795-S799.
16. Nagaraj Basavegowda, Agnieszka Sobczak-Kupiec, Dagmara Malina, Yathirajan HS KeerthiV R, Chandrashekar N, Salman Dinkar1, Liny P:2012. Plant mediated synthesis of

goldnanoparticles using fruit extracts of Ananas comosus (L.) (Pineapple) and evaluation of biological activities. Advanced Materials Letters.

17. Sunil Pandey, Goldie Oza, Arvind Gupta and Madhuri Sharon: Novel Biological Approach for Biosynthesis of Anisotropic Gold Nanoparticles using Aloe barbedensis: Role of pHand temperature. Annals of Biological Research., 2012; 3(5): 2330-2336.
18. Gopinath.S.M, *et al*., Extracellular Biosynthesis Of Gold Nanoparticles, Characterization and Medical Applications - A Review: International Journal of Advanced Biotechnology and Research., 2013; 4(2): 1127-1136.
19. Gopinath.S.M, *et al*., 2013. Biological Synthesis, Characterization and Application of Silver Nano Particles - A Review: International Journal of Pharmaceutical Applications., 2013; 4(1): 19-28.