Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Facile and Green Synthesis of Iron and Silver supported Iron Nanoparticles from Novel Plant Extract of *Aegle marmelos*: Anti-bacterial and Anti-fungal Activity.

Shikha Munjal [a], Aakash Singh*[a,b]

[a] Department of Chemistry, School of Basic Sciences, Jaipur National University, Jaipur, India

[b] Department of Chemistry, Suditi Global Academy, Mainpuri, UP, India

ABSTRACT

Nanoscience and nanoparticles are getting attention in wide aspects of Chemistry, Pharmaceutical Chemistry, biotechnology medicine and even industries. The green synthesis of different single and doped metal nanoparticles has been known for last few years and having their beneficial, medicinal and pharmaceutical result. In the light of all this, we are approaching for an ecofriendly, less perilous and plant mediated green synthesis of Iron oxide nanoparticle as compared with other conventional and unsafe methods. We report for the synthesis of iron oxide NPs by aqueous extract of leaf of variety of medicinally important plant belonging from family Rutaceae and Aegle marmelos species. During the course of study different result was observed for different part of different plants, an intense surface resonance Plasmon band in the UV-visible spectrum as the basic characterization. The further study of formation of iron oxide NPs were confirmed by FTIR, SEM,EDX, TGA and XRD.

Keywords: Green Synthesis, Rutaceae, A. marmelos extract, Ag-iron oxide nano particles.

Article Info: Received 28 April 2019; Review Completed 29 May 2019; Accepted 01 June 2019; Available online 15 June 2019

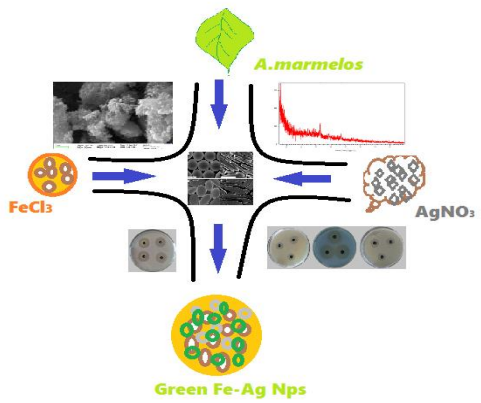
Cite this article as:

Munjal S, Singh A, Facile and Green Synthesis of Iron and Silver supported Iron Nanoparticles from Novel Plant Extract of *Aegle marmelos*: Anti-bacterial and Anti-fungal Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):334-341
http://dx.doi.org/10.22270/jddt.v9i3-s.2850

***Address for Correspondence:**

Aakash Singh, Department of Chemistry, Suditi Global Academy, Mainpuri, UP, India

Graphical Abstract:



1. INTRODUCTION

Nanotechnology deals with nanoscale ranging from 1 to 100 nm and their applications [1]. Among different type of nanomaterials, metal nanoparticles have wide range of applications and gained considerable attention due to their special catalytic, electronic, and optical properties [2] and having surface characteristics, super paramagnetic nanoparticles offer a great potential in many biomedical applications, such as cellular therapy, tissue repair, drug delivery, magnetic resonance imaging (MRI) [3].

The traditional chemical methods used for the synthesis of nanoparticles causes the formation of harmful byproducts and contamination from precursor chemicals [4]. Hence, there is growing need to develop a technology which is environmental friendly, non-toxic, green solvent, less reaction time, better atom economy and clean procedures for the synthesis of nanoparticles. Biosynthesis of metal nanoparticles by plants is presently under development and has gained great importance in current years. It is easy to handle and control the size and shape of nanoparticles. Plant-based synthesis is relatively faster, safer as well as every part of the plant is proved to be useful especially the leaves due to presence of various alkaloids, different natural bio-organic chemical moiety and their capping nature [5-9]. Nanoparticles are synthesized within few minutes or hours depending on the plant extract and methodology [10]. The bio-organic heterocycles present in plant extracts can easily reduce the metal ions in very short time.

Aegle marmelos which is commonly known as bael (or bili or bhel [11-12]), also Bengal quince [13], golden apple,

Japanese bitter orange, stone apple or wood apple belongs to the family of *Rutaceae*. *Aegle marmelos* used as medicinal plant in India traditional system of medicine the Ayruveda and its different parts are used for different health conditions. It keeps blood sugar level under control and helps to keep the intestines healthy. Bael has many benefits and uses for instance to cure tuberculosis, dysentery, constipation, useful in worm infestation and stomach related problems. It contains tannic acid, volatile oil, mucilaginous liquid and polyphenols such as alkaloids, terpenoids and phenyl propanoids which help in the reduction of metal.

The elements of group V, I and IB groups have been studied for p-type doping [14], silver as reported as the best candidate because of its high solubility, large ionic size, and minimum orbital energy [15]. Doping of iron oxide with metals such as gold, platinum, rhodium and silver increases the spectrum of radiation absorption by Fe_3O_4, allowing for its use with visible light [16]. Silver has a low cost when compared to other noble metals and has electronic properties that make it a good option for use as a dopant on the surface of Iron oxide. The effective function of silver (4.26 eV) lies below the conduction band and therefore silver can detain the photo generated electrons of Fe_2O_3 [17-19].

The present study describes a green and rapid method to synthesize the silver doped iron nanoparticles by using *Aegle marmelos* plant extract. In present research report, iron oxide nanoparticles were prepared using iron chloride as initial precursor and extract of *Aegle marmelos* leaf as reducing agent and stabilizer. The antibacterial effect of iron oxide nanoparticles was evaluated against two pathogenic bacteria, including *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) using the agar as media by well diffusion method. To the best of our knowledge, there is no work reported on adopting Aegle marmelos in the synthesis of iron oxide nanoparticles.

2. EXPERIMENTAL

2.1 Material and Methods:

All chemical are of analytical grade. Iron (III) chloride anhydrous (Merck), Ethanol (Merck), AgNO3 (Spectrochem), UV Spectrophotometer double beam Shimadzu model no 1800, FTIR Perkin Elmer Spectrum Version 10.4.00 (4000-400 cm-1 KBr) SEM Nova Nano FE-SEM 450 (FEI), XRD (Panalytical Xpert pro).

2.2 Preparation of Plant extract solution:

Fresh leaves of *Aegle Marmelos* were collected from the local area of Jaipur District, Rajasthan, India. The collected leaves were washed carefully under running water to remove the dust particle. 10 gm of fresh leaves were dried in hot air oven and then crushed well, boil it in 100 ml of water for about 2-3 hrs at 60-80˚C. The leaf extract when changes to greenish brown color, then allowed for settling down for overnight, then filter and store the extract solution below 25˚C before use.

2.3 Synthesis of Iron oxide Nanoparticles:

0.5 M solution of anhydrous Iron (III) chloride added to plant extract solution in 1:1 ratio. Fix the pH of solution at one by adding HNO_3. The acidic solution of Iron (III) chloride and leaf extract of *Aegle Marmelos* heated at 60˚C for 2 hrs under vigorous stirring. After two hours of continuous stirring, solution was allowed to settle down overnight. Remove the watery part if any and centrifuge the rest of solution at 15000 rpm for 10 min to obtain the nano-sized Fe_3O_4 particles. The collected metal nanoparticles were washed repeatedly for 2-3 times with ethanol and water, finally separated nanoparticles were dried in hot air oven at 80°c and then calcinated at 500˚C for 4 hrs in Muffle Furnace. Now the particles were grinned using mortar pestle to get the finest particle size suitable for further Characterization technique.

2.4 Synthesis of Nano Silver Iron oxide:

0.5 M solution of anhydrous Iron (III) chloride mixed with15ml of 0.1M $AgNO_3$ solution in methanol under stirring 500 rpm at room temperature for a while, equal amounts of plant extract solution mixed with silver iron (III) chloride solution and stirring again for 36 hrs at 500 rpm at room temperature without entering of light in solution, color change was observed from greenish brown to dark grey color, the product was then dried at1000°C in hot air oven, washed repeatedly for 2-3 times with large amount of ethanol and water. Nanoparticles sample were calcinated at 2500°C in muffle Furnace and then grind the sample using mortar Pestle to crush the particle into finest particle size for better characterization.

3. RESULT AND DISCUSSION

3.1 Ultraviolet spectroscopy (optical characterization):

The UV spectrum of dilute suspension of *A.marmelos* based Silver supported iron nanoparticles Nps were recorder on Shimadzu Model No.1800 double beam U.V Spectrophotometer. The absorption peak obtained in UV-Vis absorption spectra in visible range of wavelengths at 422 nm, which confirmed the presence of silver and good agreement with the theoretical results. The absorption peak of iron oxide NPs were displayed at 404.0 nm in visible range between 200 to 800 nm wavelength. Peak at 404.0 nm confirmed the formation of iron oxide (Fig 1).

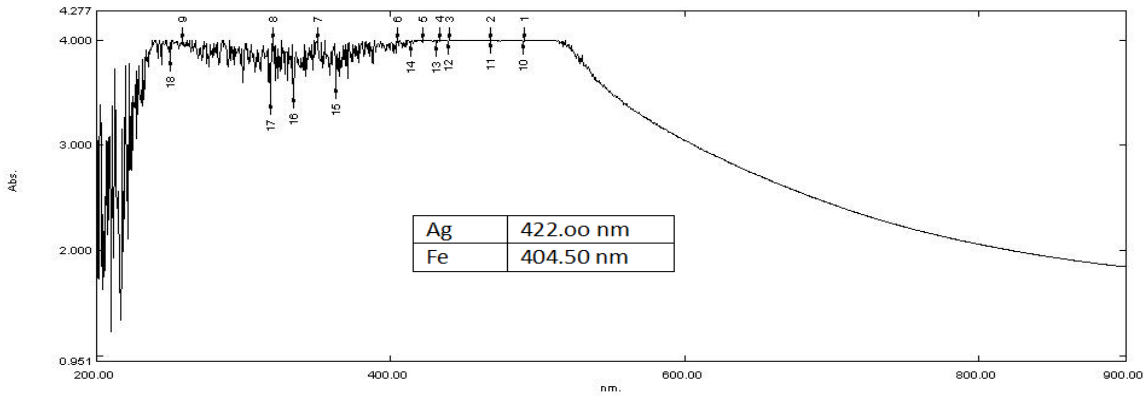


Fig 1: UV Spectrum of Ag-Fe Nps

3.2 Fourier transformation infrared spectroscopy:

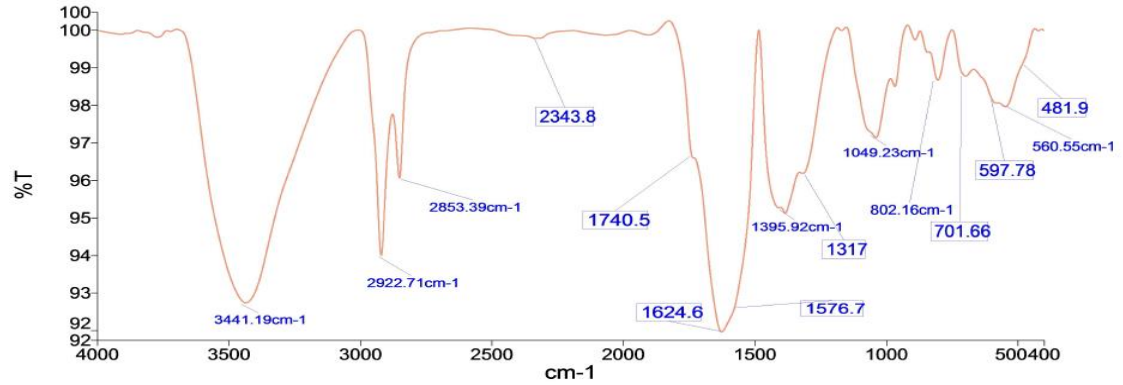


Fig- 2 FTIR of Ag-Fe NPS

Fourier transformation infrared spectroscopy (FTIR) of Ag-Fe_3O_4 nano sample was recorded from Perkin Elmer spectrum version 10.4.00 (4000-400 cm-1) using KBr pallets. In the graph at T1 line various peaks at 3441.91cm-1 (H-O-H str), 2922.41 cm-1(C-H str), 2853.30 cm-1 (C-H str), 2343.15 cm-1(C≡C and C≡N), 1740.50 cm-1 (Esters) confirms the different capping agents present in plant extract which get merged with the surface of nanoparticles where as peaks at 1624 cm-1 and 1576.7 cm-1 (silver) and 597.78 cm-1,560.55 cm-1and 481.90 cm-1 confirms the presence of iron oxide. (Fig 2)

3.3 Scanning electron microscopy and EDX:

SEM analysis shows that nanoparticles formed the cluster of aggregates at scale bar of 200nm, 300 nm and 1µm. They appeared like spherical shape with particle size less than 100 nm.(Fig 3b) The silver supported nano-particles at scale bar 200 nm confirms the nano range of particles.(Fig 3a)

The EDX spectral analysis reveals the presence of carbon, oxygen, nitrogen, iron and silver in the synthesized nanoparticles by which the presence of silver and iron was confirmed. The appearance of peak of C, N and O is due to presence of biological moieties of plant at the surface of Ag doped Fe nanostructure.(Fig 4)

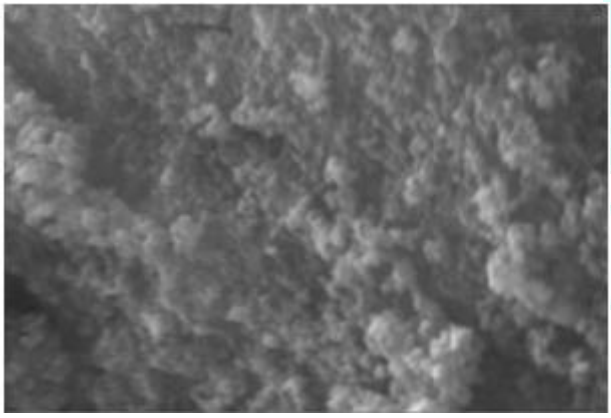


Fig 3a: SEM spectra of Ag-Fe NPS

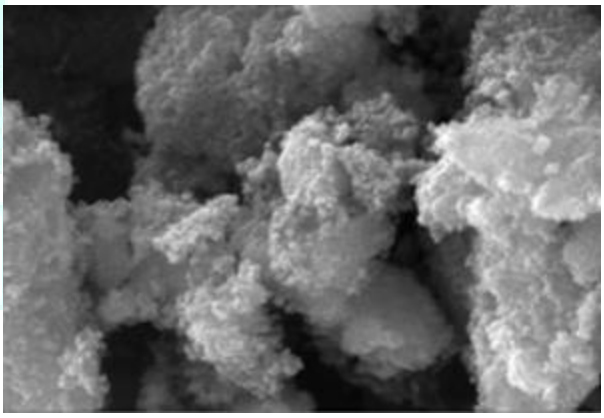


Fig 3b: SEM spectra of Fe NPS

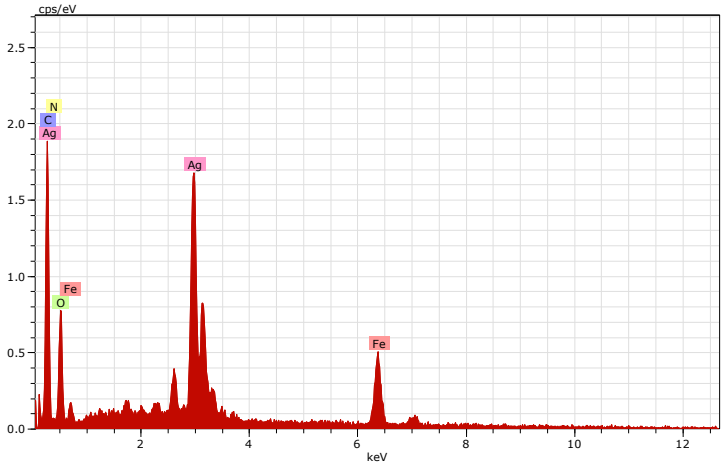
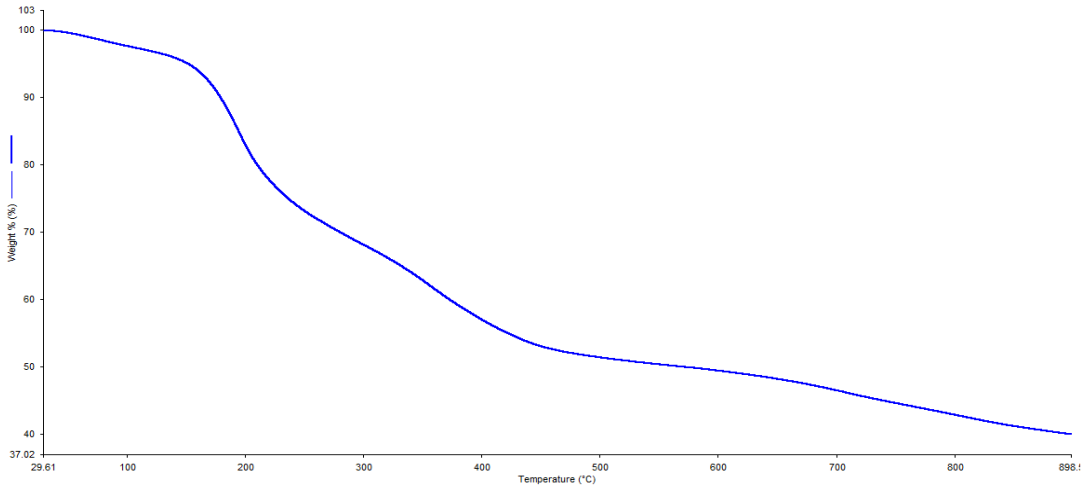
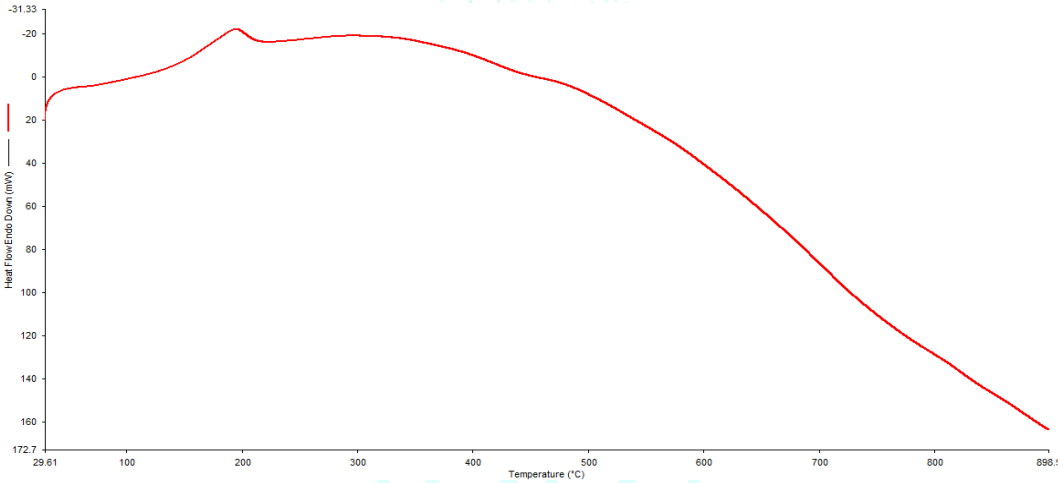


Fig 4: EDX plot showing peaks of Ag, Fe, C, N and O

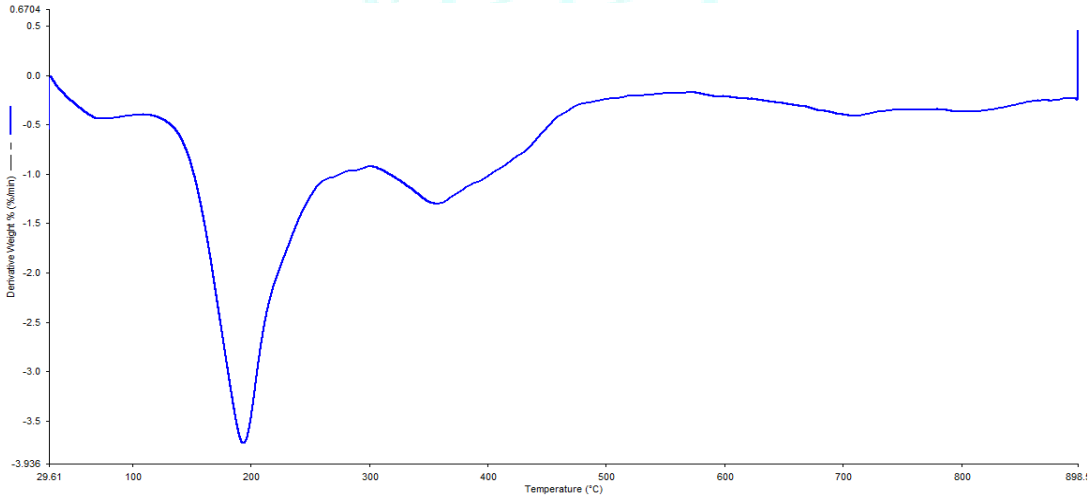
3.4 TGA: The TGA curve of Ag doped Fe nanoparticles is shown in graph 1, 2 and 3 representing weight %, heat flow and derivative weight % variation with respect to temperature ranging from 0 to 898^{0}C. The TGA graph 1 represents the continuous weight % loss of sample. This weight loss is due to water loss which absorbed by the nanoparticles during the biosynthesis, after that the sample weight is first decreasing, then constant and then decreases which reveals that temperature range 450-600^{0}C of sample is thermo stable and rest unstable.



Graph 1: Weight % versus Temperature



Graph 2: Heat flow versus Temperature



Graph 3: Derivative weight % versus Temperature

3.4 X-ray diffraction spectroscopy:-

The XRD analysis of synthesized Ag- Fe_2O_3 nanoparticles from plant extract confirms that they having black color cubic geometry of magnetite. Two distinct peak of 2Θ = 32.69 and 46.70 for iron and four distinct peaks of 2Θ = 38.32, 44.44, 64.58, 77.58 for silver were compared with the standard value (JCPDS fill no-19-0629). (Fig 5a, 5b)

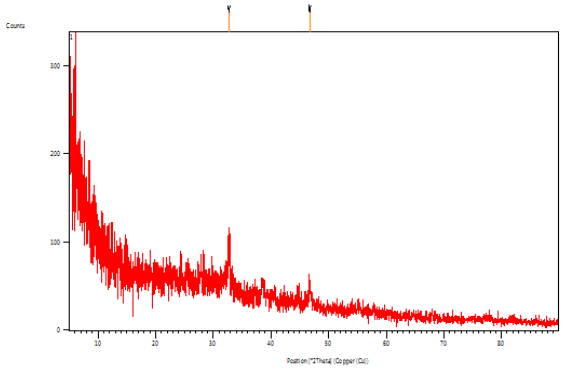


Fig (5a) XRD pattern

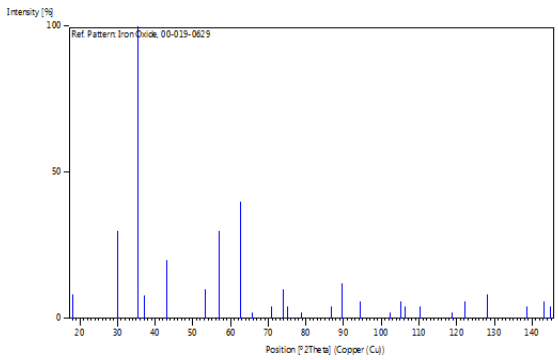


Fig (5b) XRD values of 2Θ

4. Biological Activity

4.1 Antibacterial activity:-

The well diffusion method was used to measure the antibacterial activity against model test strain of gram negative *E. coli* (MTCC 1721) and gram positive *S. aureus* (MTCC 3160). The temperature, pressure and time for sterilizing all the reagent, media and glassware are 121^{0}C, 15 PSI and 30 min respectively. The utilized bacterial concentration 1.8×10^8 CFU/ml was tested against 20 ml of three samples- Plant extract, Fe_2O_3 Nps, and Ag-Fe_2O_3 Nps at three different concentration 12.5 mg/L, 25 mg/L and 50 mg/L in three different steps.(Fig:6) The plates were incubated for 24 hrs at 37^{0}C temp.

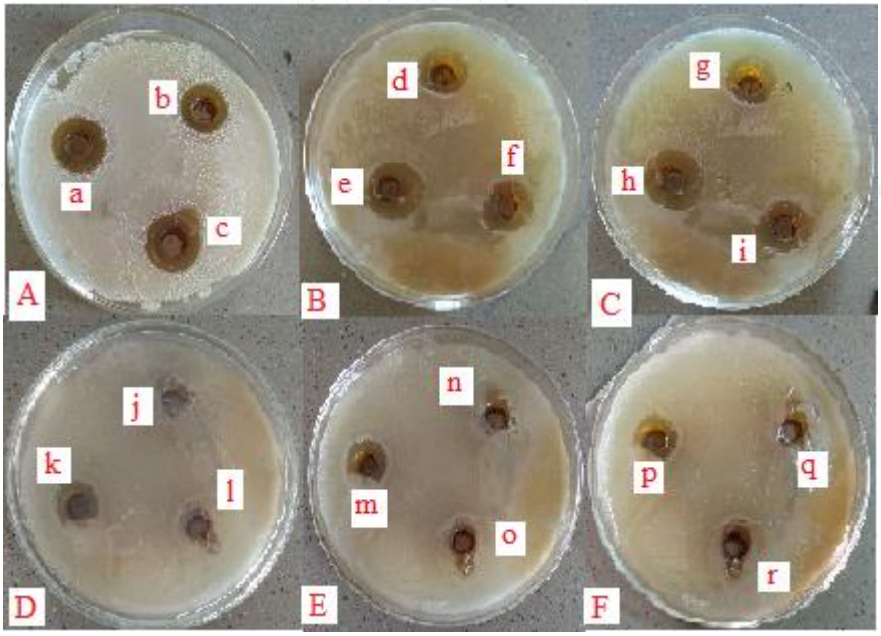


Fig: 6 Observation of Antibacterial activity at three different concentration 12.5 mg/L (**A**-*E.coli*, **D**- *S. aureus*), 25 mg/L (**B**- *E.coli*, **E**- *S. aureus*) and 50 mg/L (**C**-*E.coli*, **F**- *S. aureus*).

- a,e,h,k,m and p are zone of inhibition of *A. marmelos.*
- b,d,g,j,n and q are zone of inhibition of *A. marmelos* and Fe Nps.
- c,f,i,l,o and r are zone of inhibition of *A. marmelos* and Ag-Fe Nps.

The final result of antimicrobial activities was observed by measuring the growth of zone of inhibition diameter in mm and MIC in µgm/ml for plant extract, Fe_2O_3 Nps and Fe_2O_3-Ag Nps as given in (**Table 1**). Fe_2O_3-Ag Nps have maximum growth of zone of inhibition at various concentration 12.5, 25, 50 mg/disc. The zone of inhibition was measured as 13.53mm, 17.65mm and 20 mm respectively for *S. aureus* and comparatively less growth for *E. coli* was found to be 11.50mm, 15.43mm and 18.90 mm respectively. The result of Antibacterial activity revealed that of Ag doped iron oxide nanoparticles observed to be more active than plant extract and Fe_2O_3 and was more potent against *S. aureus* with 50mg/l value.(Fig 7 a,b and c)

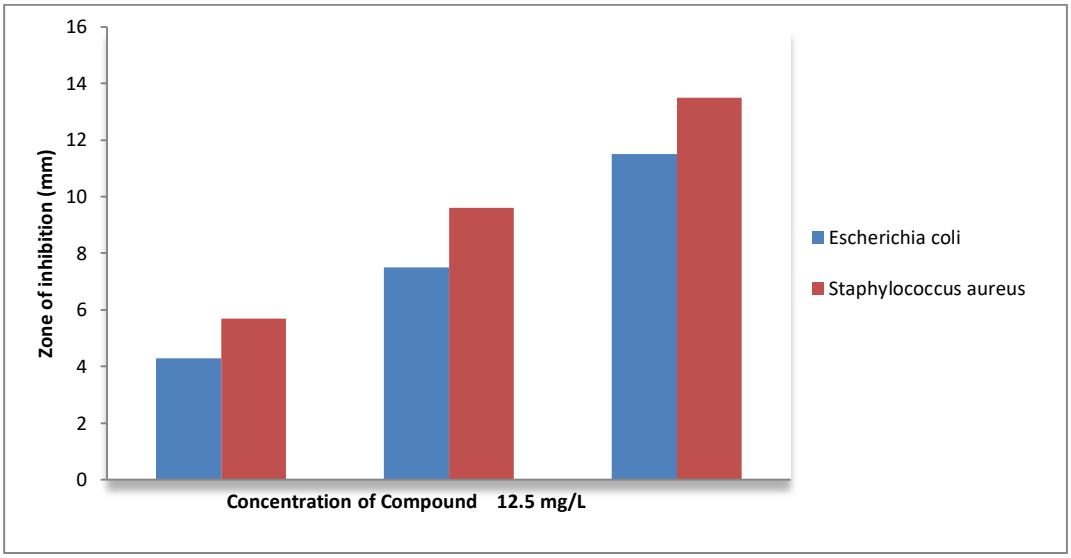


Fig 7(a): Comparison of Antibacterial activity of *E.coli* and *S. aureus* at conc. 12.5 mg/L

Table 1: Observation of antibacterial activity

S.N	Name of Bacteria	Test Sample	Concentration	Zone of inhibition (mm)
1	*E. coli* (MTCC 1721)	*A.marmelos* extract	12.5 mg/L	4
			25 mg/L	4
			50 mg/L	6
2	*E. coli* (MTCC 1721)	Fe_2O_3 Nps	12.5 mgLl	7.5
			25 mg/L	6
			50 mg/L	11
3	*E. coli* (MTCC 1721)	Fe_2O_3-Ag Nps	12.5 mg/L	11.5
			25 mg/L	16
			50 mg/L	18
4	*S. aureus* (MTCC 3160	*A.marmelos* extract	12.5 mg/L	5.5
			25 mg/L	6
			50 mg/L	7.5
5	*S. aureus* (MTCC 3160	Fe_2O_3 Nps	12.5 mg/L	9.5
			25 mg/L	8
			50 mg/L	12.5
6	*S. aureus* (MTCC 3160	Fe_2O_3-Ag Nps	12.5 mg/L	13.5
			25 mg/L	18
			50 mg/L	20

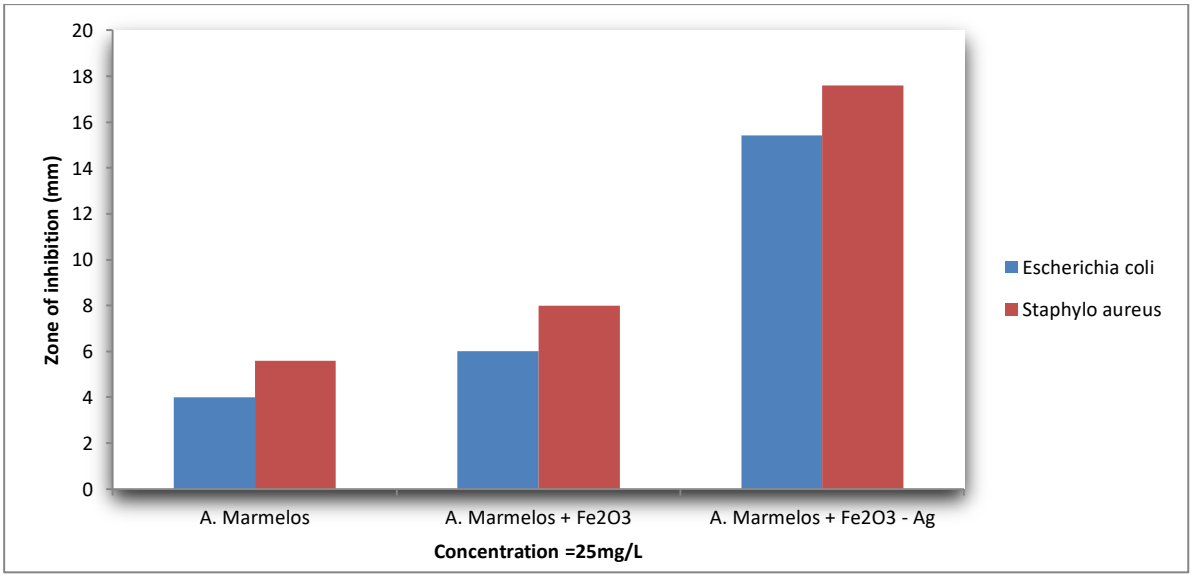


Fig 7(b): Comparison of Antibacterial activity of E.coli and *S. aureus* at conc. 25 mg/L

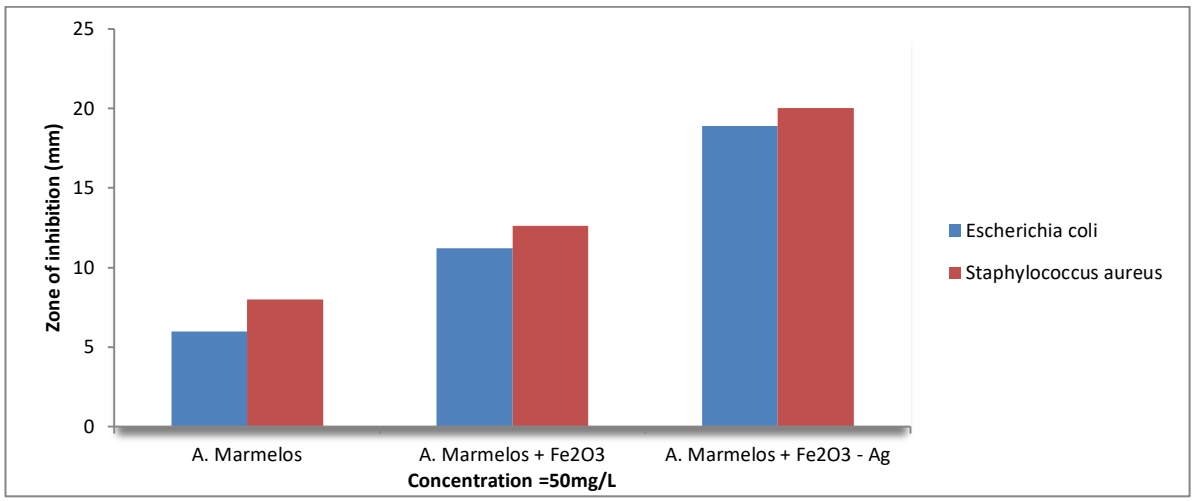


Fig 7(c): Comparison of Antibacterial activity of *E.coli* and *S. aureus* at conc. 50 mg/L

4.2 Antifungal activity:-

The Antifungal activity of *A. marmelos* plant extract, Fe nano and Ag-Fe_2O_3 nano particles to be tested against *Aspergillus niger* fungus by well diffusion method on the surface of PDA (Potato Dextrose Agar)media incubated with 1×10^5 CFU/ml of spore suspension of *Aspergillus niger* fungi.

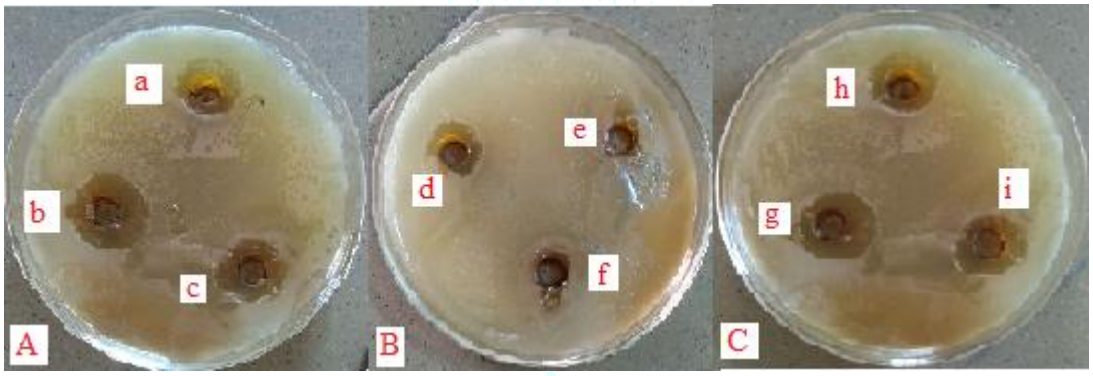


Fig: 8 Observation of Antifungal activities at three different concentration (**A**- 100 ppm, **B**- 500 ppm, and **C**- 1000 ppm).

- (a),(e) and (h) represents zone of inhibition of *A.marmelos*
- (b),(d) and (g) represents zone of inhibition of *A.marmelos* and Fe Nps.
- (c),(f) and (i) represents zone of inhibition of *A.marmelos* and Ag-Fe_2O_3 Nps.

The potato dextrose broth (PDB) was prepared and placed in shaking incubator for 48 hrs and then *Aspergillus niger* fungus were suspended into it. Potato Dextrose Agar (PDA) is allowed to set and were uniformly seeded with *Aspergillus niger* fungus taken from suspension.

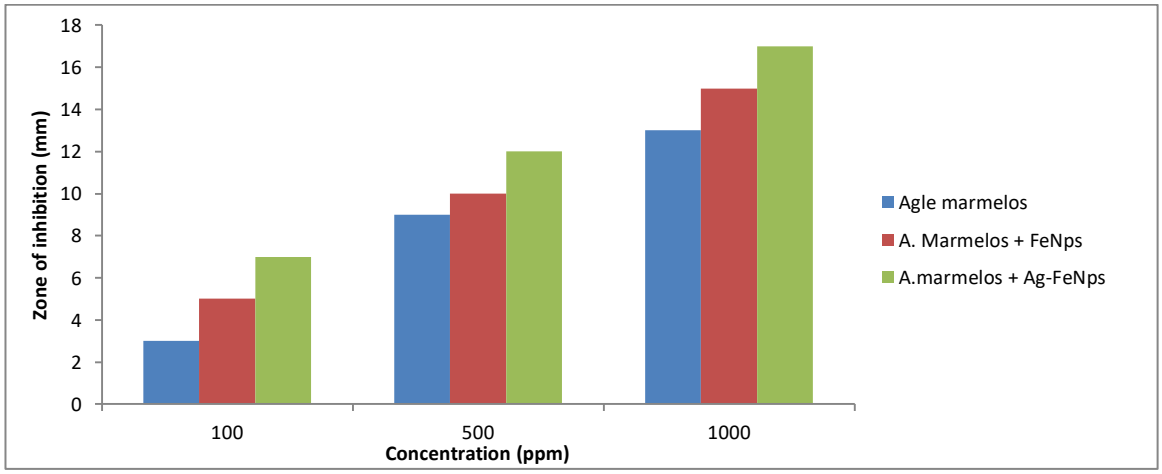


Fig 9: Comparison of Antifungal activity of Aspergillus *niger* at conc. (100ppm, 500 ppm, 1000 ppm).

Small wells having 6mm diameter impregnated with the solution of *A. marmelos* plant extract, Fe nano and Ag-Fe_2O_3 nano particles were poured into the plates of culture medium at different concentration of 100, 500 and 1000 ppm. Plates were immediately transferred to incubator for 48 hrs at 25-28^{0}C temp. After 48 hrs of incubation, degree of sensitivity is determined by measuring the zone of inhibition in mm. (**Table 2**). The increasing ppm concentration is directly proportional to activity against *Aspergillus niger.* The best activity was shown by Ag-Fe_2O_3 Nps of *A. marmelos* at 1000 ppm concentration.

Table 2: Observation of antifungal activity.

S.N	Name of Fungi	Concentration (ppm)	Test Sample	Zone of inhibition (mm)
1	*Aspergillus niger*	100	*A.marmelos* extract	3
			Fe_2O_3 Nps	5
			Fe_2O_3-Ag Nps	7
2	*Aspergillus niger*	500	*A.marmelos* extract	8.5
			Fe_2O_3 Nps	10
			Fe_2O_3-Ag Nps	12
3	*Aspergillus niger*	1000	*A.marmelos* extract	12.5
			Fe_2O_3 Nps	14.5
			Fe_2O_3-Ag Nps	16.5

5. CONCLUSIONS

In summary, we have developed convenient and green procedure for the synthesis of Fe_2O_3 and Ag- Fe_2O_3 from novel plant *Aegle Marmelos* for the first time. The employed plant plays important role as capping agent on the surface of metal nanoparticles of iron and silver.

ACKNOWLEDGEMENTS

We are thankful to the MRC, MNIT, Jaipur for the spectral analyses. We are also thankful to the Life science Department and Chemistry Research Laboratory of Jaipur National University.

REFERENCES

1. M. Zargar, A. A. Hamid, F. A. Bakar et al., "Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L.," *Molecules*, vol. 16, no. 8, pp. 6667–6676, 2011.
2. K. J. Rao and S. Paria, "*Aegle marmelos* leaf extract and plant surfactants mediated green synthesis of Au and Ag nanoparticles by optimizing process parameters using taguchi method,"*ACS Sustainable Chemistry and Engineering*, vol. 3, no. 3, pp. 483–491, 2015.
3. J. Guoa, R. Wanga, W.W. Tjiu, J. Pan, T. Liu, Synthesis of Fe nanoparticles@graphene composites for environmental applications. *J. Hazard. Mater.* vol. *225–226*, pp. 63–73, 2012.
4. K. N.Thakkar, S. S. Mhatre, and R.Y. Parikh, "Biological synthesis of metallic nanoparticles," *Nanomedicine*, vol. 6, no. 2, pp. 257–262, 2010.
5. I. M. Chung, I. Park, K. Seung-Hyun, M. Thiruvengadam, and G. Rajakumar, "Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications," *Nanoscale Research Letters*, vol. 11, no. 1, pp. 1–14, 2016.
6. D. Philip, "Rapid green synthesis of spherical gold nanoparticles using *Mangifera indica* leaf," *Spectrochimica Acta Part A:Molecular and Biomolecular Spectroscopy*, vol. 77, no. 4, pp. 807–810, 2010.
7. S. S. Dash, B. G. Bag, and P. Hota, "*Lantana camara Linn* leaf extract mediated green synthesis of gold nanoparticles and study of its catalytic activity," *Applied Nanoscience*, vol. 5, no. 3, pp. 343–350, 2015.
8. V. G. Kumar, S.D. Gokavarapu, A. Rajeswari et al., "Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent *Cassia auriculata*,"*Colloids and Surfaces B: Biointerfaces*, vol. 87, no. 1, pp. 159–163, 2011.
9. B. Sadeghi, M. Mohammadzadeh, and B. Babakhani, "Green synthesis of gold nanoparticles using *Stevia rebaudiana* leaf extracts: characterization and their stability," *Journal of Photochemistry and Photobiology B: Biology*, vol. 148, pp. 101–106, 2015.
10. M. Rai, A. Yadav, and A. Gade, "CRC 675—current trends in phytosynthesis of metal nanoparticles," *Critical Reviews in Biotechnology*, vol. 28, no. 4, pp. 277–284, 2008.
11. "FOI search results". flowersofindia.net. Retrieved 20 January 2016.
12. G.P. Wilder, (1907), Fruits of the Hawaiian Islands, Hawaiian Gazette, ISBN 9781465583093.
13. "Aegle marmelos". Germplasm Resources Information Network *(GRIN).* Agricultural Research Service *(ARS),* United States Department of Agriculture *(USDA)*. Retrieved 20 June 2017.
14. D. Sahu, N. Panda, B. Acharya, A. Panda, Enhanced uv absorbance and photoluminescence properties of ultrasound assisted synthesized gold doped zno nanorods, Optical Materials vol. 36, no. 8, pp. 1402–1407, 2014.
15. Y. Yan, M. Al-Jassim, S. H. Wei, Doping of zno by group-ib elements, Applied
Physics letters vol. 89, no.18, pp.181912–181915, 2006.
16. M. M. Gui, W. M. P. Wong, S. P. Chai, A. R. Mohamed, *Chem. Eng. J.* vol 278, pp. 272-278, 2015.
17. L. G. Devi, B. Nagaraj, K. E. Rajashekhar, *Chem. Eng. J.* vol. 259 pp. *181-182*, 2012.
18. N. Sobana, K. Selvam, M. Swaminathan, *Sep. Purif. Technol.* Vol 62, no. 3, pp. 648-653, 2008.
19. J. M. Stropa, A. S. Herrero, S. C. Oliveira, A. A. Cavalheiro, F. Dantas, S. L. Oliveira, L. C. S. Oliveira, *J. Braz. Chem. Soc.*vol 27 no 3, pp 575-580, 2016.