



## SYNTHESIS OF Ag-Fe BIMETALLIC NANOPARTICLES USING *ROSMARINUS OFFICINALIS* LEAF EXTRACT

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### ABSTRACT

Simple and ecofriendly method was used for synthesizing Ag-Fe bimetallic nanoparticles at room temperature. The reducing agent was rosemary extract which competed with silver nitrate (AgNO<sub>3</sub>) and ferric chloride (FeCl<sub>3</sub>) solution in the formation of Ag-Fe nanoparticles. The formation of highly stable Ag-Fe nanoparticles at room temperature was easy through the use of leaves of the *Rosmarinus officinalis*. Using UV spectroscopy, the crystalline phase and morphology of the bimetallic Ag-Fe Nps in which the absorption band at 450 nm was identified. The Fourier transform infrared spectroscopy (FT-IR) was performed and the functional groups that cause the bio-reduction of silver ion and ferric ion were identified. Thermogravimetric analysis (TGA) was used to determine the weight loss of the Ag-fe alloy Nps and found that the sample could withstand heat up to 900 °C. To analyse the crystalline nature of bimetallic, X-ray diffraction was carried out and the average size of crystalline determined using Scherrer equation and was found to be 17 nm. The antimicrobial effect of Ag-Fe alloy nanoparticles demonstrated that the nanoparticles can be utilized as effective growth inhibitors to *Escherichia coli* and *Bacillus Substills*. Photocatalytic experiment demonstrated the capability of the catalyst produced to degrade a pollutant dye, Bromothymol Blue.

### 1. Introduction

Nanotechnology is the most promising field of study in the contemporary material science and it is significantly contributing in biotechnology and biomedical research. The properties of nanoparticle are totally new because they include certain properties like shape, size, and distribution [i,v]. There are various ways of biosynthesizing nanoparticles like electrochemical, radiation aspired, reduction in solution, microwave-aided route and recently green chemistry approach [vi]. Plant extracts (leaves, fruit peels flower, seed, stem bark, etc.) synthesis also has several advantages in terms of biomedical applications which do not require

the use of lethal chemicals in the synthesis process, and also may provide a higher yield of well-shaped and size nanoparticles [vii,viii].

Silver nanoparticles find extensive application in a broad range of viable products as antimicrobial agents (because of broad range and low cytotoxicity) in those products are used in various fields, e.g., household (antibacterial coating, hand sanitizer, sterilizing clothes etc.), electronic, medical, drinking water treatment, and nanomedicines [x].

In most cases nano particles exhibit excellent antimicrobial, antifungal, catalytic activity in a good extent thus has generated a great number of researchers. Such activities can be enhanced to a greater degree by synthesis of bimetallic nanoparticles. They can be synthesized using plant extracts and rank as a promising study of these bimetallic nanoparticles because it is cheaper, meeker, innocuous, faster, and easier than conventional methods that can be employed. Silver-iron bimetallic nanoparticles, in turn, have a range of applications in the areas of optics, medicine, remediation.

In 2018 Aisha Al-Asfar and colleagues have prepared Ag-Fe bimetallic nanoparticles of Palm dates fruit through green synthesis method and have utilized them as catalysts to degrade bromothymol blue in effect of sunlight [viii]. Ag-Fe bimetallic synthesis by one-pot green method Maqsood A. M. ed. al. used aqueous extract of *Salvia officinalis*, the catalytic activity was investigated in the reduction of 4-nitrophenol [ix].

Co-combination of Ag NPs with other metal to form an alloy is also a significant approach to developing stability and biocompatibility of Ag NPs. A huge number of studies have been published to prepare bimetallic nanomaterial like Au-Ag, Au-Pt, Ni-Pd, Cu-Ag [xi, xviii] and Pt-Ag [xii]. Synthesis of Ag-Fe alloy nanostructure has very few reports that have been studied. In addition to biological applications, it has been used that Fe Ag nanoparticles can be utilized in degradation of water pollutant dyes. Gallo and team have reported the Synthesis of bimetallic silver/iron nanoparticles to treat water and evaluate its reactivity on bromophenol blue [xiii-xvii].

Thus, in this paper, Ag-Fe bimetallic nanoparticles were synthesized by using redox process with the help of the reducing agent *R. officinalis* leaf extract. Characterization of synthesized nanomaterial was carried out using XRD, FT-IR, TGA, UV-visible, and microbial activity was done using the pathogenic strains. Bromothymol blue was also studied in a photocatalytic manner. The subsequent study had shown the capability to apply green synthesis of magnetic bimetallic nanoparticles made of Ag and Fe and had shown that they have synergistic effect in antimicrobial action. Besides, the results that are outlined here give some directions to the development of new antimicrobial pharmaceuticals that are stronger and have multiple uses and also in the possible treatment of the contagions caused by the clinically significant drug-sensitive and drug-resistant organisms.

## 2. Material and methods

### 2.1 Materials

Silver nitrate ( $\text{AgNO}_3$ ) and ferric chloride ( $\text{FeCl}_3$ ) were acquired at Merck, Germany. All the stock solutions were prepared using the double distilled and deionized and CO free water. The leaves of *Rosmarinus Officinalis* commonly referred to as common sage were bought in a nearby market in Pune, India.

### 2.2. Plant extracts preparation

The freshly dried rosemary leaf was adequately washed by several times using distilled water so as to get rid of any impurities on the surface of the plant. These rosemary leaves were first dried in the room temperature and crushed into powder. The leaf powder (10 mg, 50mg,

100mg and 1 g) was added into 50 ml of double distilled water in 500 ml of Erlenmeyer flasks and the flasks were boiled under the constant stirring to prepare the different concentration of rosemary extract, which was used in the synthesis of Ag-Fe nanoparticles.

### 2.3 Agglomeration of nanoparticles -Synthesis of Ag-Fe alloy nanoparticle

The synthesis of Ag-Fe alloy nanoparticles was conducted in the form of a green synthesis at room temperature. The solution of 100 ml of metal (Fe) ion was combined with 100 ml. of the solution of metal (Ag), and the solution was reduced in the presence of varying concentration of the rosemary extract at room temperature and the changes of the solution were noted.

All flasks were taken UV - Scan, and then all flasks were placed in room temperature (until nanoparticles precipitated). The flasks were centrifuged after 10 min. Nanoparticles precipitate more in the flasks with less concentration of the sample (10 ml. of 50mg rosemary extract). The further analysis of these nanoparticles was done.

## 3. Results and Discussion

### 3.1 Characterization of Nanoparticles

Nanomaterials such as bimetallic nanoparticles have many applications of interest. Bimetallic nanoparticles with a combination of two metals are able to exhibit a broad spectrum of characteristics because of the synergistic effect of the two different metals. The effect has the capacity to improve their attributes and capabilities that can expand our focus on their applications as antibacterial agents, drug delivery systems, and imaging agents. Our study accordingly commenced with synthesizing Ag-Fe bimetallic NPs with the help of leaf extract of rosemary then proceeded to physical-chemical characterization and their application as antimicrobials with different methods of analysis.

#### 3.1.1 FT-IR analysis of Ag-Fe nanoparticles:

FT-IR analysis was conducted, to determine the potential biomolecules that were also capping and reducing agent of the Ag-Fe bimetal nanoparticles by plant extract. With FTIR spectroscopy, the functional and composition of Ag-Fe alloy Nps was determined in the 4000-280 $\text{cm}^{-1}$ . In fig. 1 the peaks of 3130 $\text{cm}^{-1}$  and 2987 $\text{cm}^{-1}$  by the N-H and O-H stretching mode in the bonding of the proteins. The medium intense band at 1635 $\text{cm}^{-1}$  was C=O stretch occasioned by aromatic ring of various phenolic compounds in extract, the peaks at 1557 $\text{cm}^{-1}$ , 1530 $\text{cm}^{-1}$  and 1320 $\text{cm}^{-1}$  were C-C stretching vibrations, the peaks at 660 $\text{cm}^{-1}$  was C-H stretching.

The FT-IR spectroscopic analysis indicated that there is protein as well as the carbohydrate and phenols that can form a layer over the Ag-Fe alloy to prevent the agglomeration and stabilizing of the prepared nanoparticles (Fig.1).

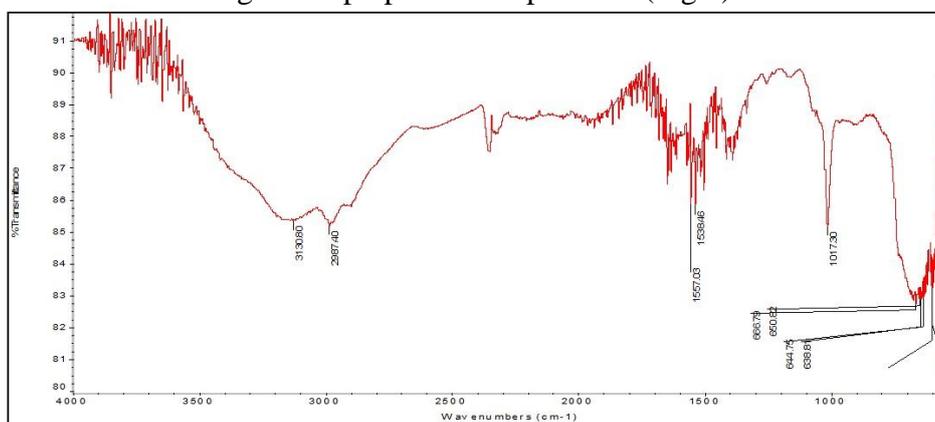


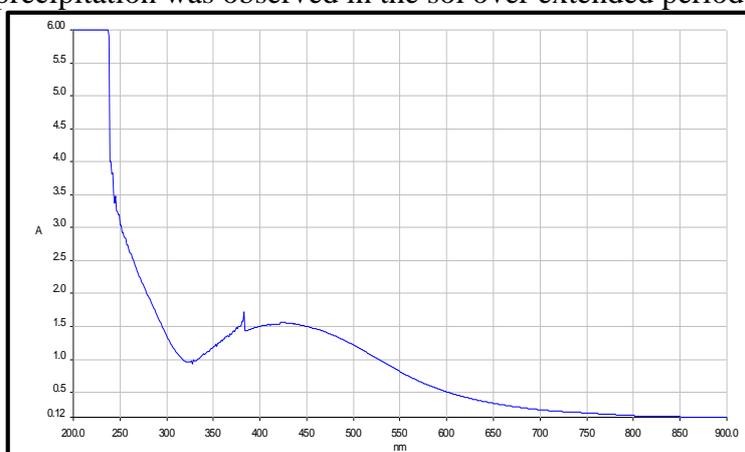
Fig.1: FT- IR-spectra of Ag-Fe alloy nanoparticles

### 3.1.2 UV-visible study of Ag-Fe nanoparticles:

UV-Vis absorption double beam spectrophotometer at 200 -900nm at room temperature was used to analyse the optical property of the synthesised bimetal nanoparticles using deuterium and tungsten iodine lamp. As the mixture was added with the Rosmarinus extract the solution of the silver and Iron metal ion, the mixture became dark greenish brown, which proved the presence of Ag-Fe alloy nanoparticles.

The UV-vis absorption spectrum of the Ag-Fe nanoparticles that were synthesized was depicted in Fig.2. Ag-Fe alloy nanoparticles contain free electrons that appear as surface plasma resonance (SPR) absorption band as a result of the joint vibration of the electrons of the silver nanoparticles in interaction with light wave. At 440 nm a wide hump was seen and this is a typical band of the alloy. The spectrum did not show any other peak which proved that the products that have been synthesized was Ag-Fe only.

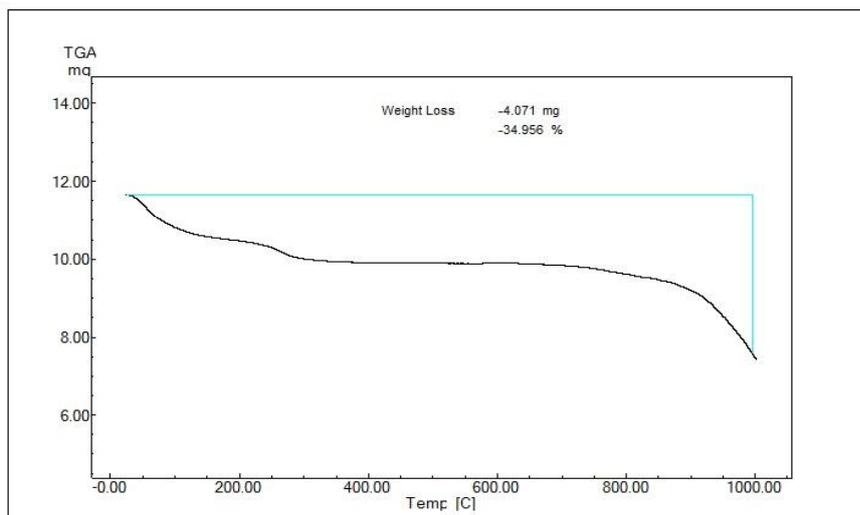
The UV-visible spectroscopy is a helpful technique that can be used to provide data regarding the shape, size, and size distribution of the metal NPs. The as-prepared Ag-Fe bimetallic nanoparticles were found to be stable in that the formation of the precipitate was followed and no precipitation was observed in the sol over extended periods of time [viii].



**Fig. 2 : UV-vis spectra of Ag-Fe Nanoparticles**

### 3.1.3 TGA analysis of Ag-Fe nanoparticles:

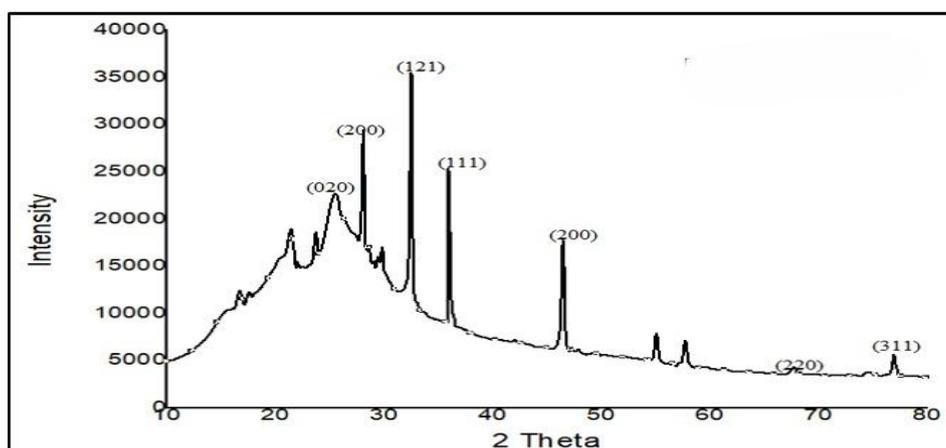
Ag-Fe alloy nanoparticles were prepared and thermogravimetric analysis was used to examine the thermal stability of the capping agents that were used on the surface of Rosmarinus Officinalis. TGA was performed in nitrogen atmosphere and the heating rate was 10 °C/min. The temperature range of 50 °C to 250 °C that corresponds to the loss of water molecules within the surface and the interstitial locations of the Ag-Fe nanoparticles was chosen to initiate the weight loss process as shown in curves (Fig. 3), no further weight loss was recorded and the stability temperature was then initiated at temperature 270-900°C which indicated that the prepared Ag-Fe alloy by the Rosmarinus Officinalis extract is thermally stable at temperatures up to 900 °C.



**Fig. 3: TGA of Ag-Fe nanoparticles**

### 3.1.4 XRD analysis

XRD analysis Fig.4 shows the crystal nature and lattice properties of the prepared nanoparticles of silver- Iron alloy by measuring powder X-ray diffraction. The aqueous extract of rosemary produced XRD pattern of Ag-Fe nanoparticles, which spontaneously exhibited the following diffraction peaks at 2 $\theta$  value of 38.1°, 44.2°, 64.4°, and 77.4°, respectively, (111), (200), (220) and (311) are the respective phases of the face-centered cubic (fcc) phase of Ag particles. The XRD pattern of Ag-Fe bimetallic nanoparticles has diffraction peaks that are comprised of the standard peaks of Ag (JCPDS no. 00-004-0783) and (020) and (121) of Fe showing the existence of bimetallic phases between Ag and Fe.



**Fig. 4: XRD of Ag-Fe alloy nanoparticles**

### 3.2 Antimicrobial activity of bimetallic nanoparticles

Prepared bimetallic nanoparticles were tested on the basis of their antibacterial activity by the measured zone of inhibition. *B. subtilis* and *E. coli* were the Gram-positive and Gram-negative bacterial models used in this study respectively. The antimicrobial activity of Ag-Fe alloy nanoparticles against *E. coli* and *B. subtilis* was studied using the well diffusion technique. The bacterial suspension was placed on a uniform Muller Hinton agar (MHA) plate with concentration of 0.025 mg/ml, 0.5mg/ml and 1mg/ ml. In the case of antimicrobial study, Ag/Fe nanoparticles colloidal solution was prepared 1:1 DMSO solvent in above

concentration and subsequently added onto the well which had been prepared, the DMSO was used as a control and the plates were incubated at 37°C 24h. The areas surrounding the wells are restricted easily. In the case of *B. subtilis* and *E. coli*, the mean of the diameter of the inhibitory zone was determined and noted to be 1.1 mm and 2.0 mm respectively. The silver-iron alloy is the possible product of rosemary leaf extract which can be used as antibacterial material, based on the determination of inhibition zone. The difference in cell walls or cell membrane structure of the microorganisms seems to be one of the essential elements in antibacterial action of Ag-Fe bimetallic NPs. The antibacterial effect of Ag-Fe bimetallic nanoparticles has not been well comprehended yet. But, core-shell nanostructures have a stronger impact compared to monometallic nanoparticles [xiv, xv]. Porins can also be used as a transport path of nanoparticles in and out of cells [xix]. It is known that silver nanoparticles can also penetrate through the bacterial cell wall and bind to it. After getting in they assist in the generation of the free radicals that lead to intracellular oxidative stress ultimately leading to cell death [xvi] Recent studies have proposed that some of the amino acids (-SH groups of cysteine) present in the proteins of the bacteria cell wall can contact with iron. It has been discovered that the thiol fraction of cysteine in specific has been the most receptive to the assault of electrons by the oxidising species. [viii] Evidently observe the inhibiting areas around the wells. The mean diameter of the inhibiting zone was determined of both *B. subtilis* and *E. coli* that had a 1.1 mm and 2.0 mm respectively. The result of the measures on the inhibition zone demonstrates that the silver-Iron alloy produced using the extract of Rosemary leaves can be used as potential antibacterial materials. Degradation of bromothymol blue in the presence of photocatalysts. To determine the catalytic activity of Ag-Fe, bromothymol blue (BTB) was used as a model of water contaminant organo-metalloid. The reaction was run in a beaker at room temperature and mixed using a magnetic stirrer under uv light and the reaction mixture contained 10 ppm bromothymol blue and 10 mol% AgFe catalyst that was activated at 110 degC. H<sub>2</sub>O<sub>2</sub> was taken as oxidizing agent. When the reaction had been completed, the photocatalyst was filtered off. The UV spectroscopy on the resulting reaction mixture was then carried out (Fig.5)

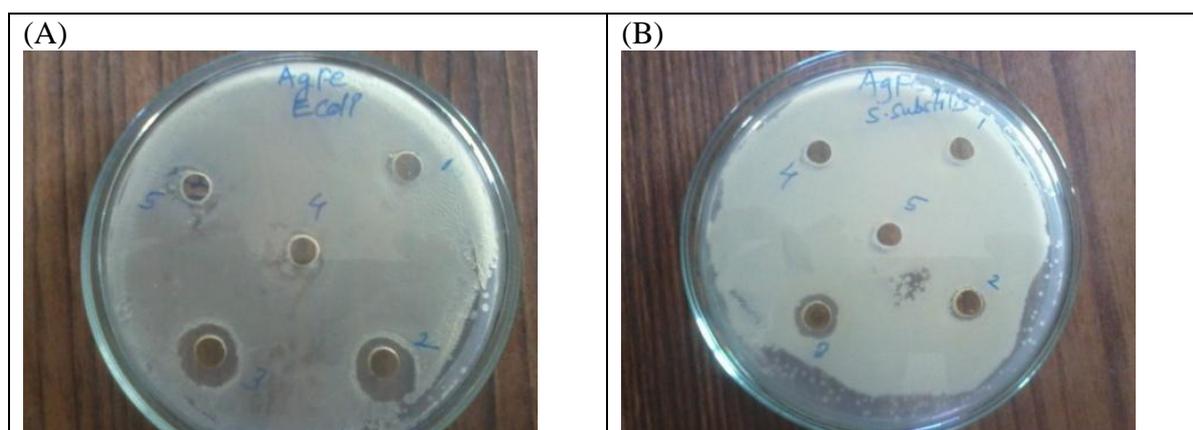


Fig.5- Showed antimicrobial activity for Ag-Fe alloy nanoparticles

### 3.3 Degradation of bromothymol blue in the presence of Ag-Fe nanoparticles:

To determine the catalytic activity of Ag-Fe, bromothymol blue (BTB) was used as a model of water contaminant organo-metalloid. The reaction was run in a beaker at room temperature and mixed using a magnetic stirrer under UV light and the reaction mixture contained 10 ppm bromothymol blue and 10 mol% Ag-Fe catalyst that was activated at 110 degC. H<sub>2</sub>O<sub>2</sub> was taken as oxidizing agent. When the reaction had been completed, the photocatalyst was filtered off. The UV spectroscopy on the resulting reaction mixture was then carried out.

#### 4. Conclusions

The alloy nanoparticles produced were further characterized using other measurements like XRD FT-IR, TGA and UV-visible Spectroscopy which confirmed the phase of the material as face-centered cubic and size as 42.8 and 41.2 nm. Bimetallic nanoparticles are also being prepared sustainably with natural extracts to be used both in the environment and in medical practice. The reducing agent was Rosemary leaves extract in the present study to produce Ag-Fe bimetallic nanoparticles and also The antimicrobial activity of bimetallic NPs was tested and it was discovered that it is synergistic against Gram-positive bacteria and Gram-negative bacteria. In our results it is revealed that green synthesis methods can be applied to make the magnetic bimetallic nanoparticles composed of metals which complement each other in terms of their antibacterial effects. Also, this work illuminates on the development of new and better antimicrobial compounds that can be employed to treat drug-resistant diseases besides to other biological uses. The photocatalytic study showed that the degradation of dyes contained in the industrial effluents can be done using FeAg nanomaterial in a good extend.

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