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Research Article

Biosynthesis Of Silver Nanoparticles with The Help of Fermented Rice Water Extract Having Antimicrobial and Antioxidant Activity

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ABSTRACT

This study presents the successful application of fermented rice water (FRW) extract in the renewable bioinspired green synthesis of silver nanoparticles (AgNPs). The green production of silver nanoparticles (AgNPs) was successfully accomplished using the aqueous-alcoholic extract of fermented rice. Analysis, FT-IR, XRD, FE-SEM, UV-Vis, and other physicochemical methods were used to characterize the green produced AgNPs. Plasmon resonance spectra with a maximum at 390 nm were evident in the UV-vis spectrum, and XRD verified the crystalline nature of FRW-AgNPs. FE-SEM analysis of the particles' size, shape, and morphology revealed that they were spherical in shape, with an average diameter of roughly 26 nm. FTIR was used to identify the presence of phenols or alcohols, carboxylic acids, ketones, and amine functional groups. According to in vitro biological activity investigations, the synthesized FRW-AgNPs have dose-dependent antioxidant activity against DPPH (75.2 ± 0.057 %) and possible antibacterial activity against Gram (+)ve and Gram (-)ve bacteria.


INTRODUCTION

Nanotechnology is currently the most exciting subject of study in contemporary material science, and it directly influences research in the life sciences. The use of biological material, such as plant extracts, fungi, bacteria, and algae, in the biogenic synthesis of nanoparticles offers many

advantages, including long-term stability of the synthesized nanoparticles and compatibility in pharmaceuticals and other biomedical applications. Green chemistry, or environmentally friendly routes, have been suggested recently due to the avoidance of harmful chemicals in the process.[1] Applications of nanotechnology in

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various domains of life, such as medicine, agriculture, the environment, food, electronics, mechanics, and the space industry, are highly remarkable. Nanoparticles generally have a size range of 1-100 nm and have unique biological, optical, catalytic, electrical, and magnetic properties owing to their small size and high surface area. Among the various nanoparticles, silver has been comprehensively studied mainly because of its broad-spectrum antimicrobial activity.[2] Due to the same, many bioresources have been exploited for greener and sustainable synthesis of AgNPs. Due to the easy availability, numerous studies have described the role of fermented rice water extract. One of the widely available sources of antioxidants is rice. In medical applications, many reports demonstrate their biological activities, such as antioxidant, and antimicrobial activities. AgNPs have been used as antibacterial agent for many kinds of applications.[3] Rice (*Oryza sativa* L.) is a staple food crop and is available in white (most popular), red, purple, black and brown color in different varieties. In this study, fermented rice water extract derived from white rice was selected for the formation of AgNPs. Fermented rice water extract was prepared by adding specific amount of DI water in some amount of rice and rest aside up to 12 to 24 hrs. for fermentation. further extract was made by filtration process [4,5]. The process was made highly rapid by exposing to sunlight irradiation. The fermented rice water extract-AgNPs were characterized by UV-vis spectrophotometric analysis, FE-SEM, FT-IR and XRD techniques.[6]

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Major chemicals, such as Silver nitrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical were purchased from SRL Chemicals (India) and

AgNO₃ from Research lab fine chem. industries. The microbial cultures were obtained from the (MTCC)- Chandigarh. All other chemicals used in the study were obtained from SD fine chemicals and SRL Chemicals (India) unless otherwise noted in the specific experimental steps. The deionized water used in the study was sourced from our own DI water system.

2.2. Rice Collection

Rice (*Oryza sativa* L.) is a staple food crop and is available in white (most popular), red, purple, black and brown color in different varieties. This type of rice was collected from local market and used as a main ingredient in silver nanoparticles synthesis.

2.2.1. Extract Preparation

About 10gm of Rice (*Oryza sativa* L.) rice was weigh on weighing balance. wash thoroughly and add 100ml of deionized (DI) water. Transfer it to a container and rest it up to 12 to 24 hrs for the fermentation process at room temperature. After 12 to 24hrs filter above mixture filter with Whatman's filter paper to avoid particles interruption and gets a cloudy solution which is a fermented rice extract.

2.3. Silver nanoparticles synthesis

2.3.1. Preliminary evaluation of silver nanoparticle synthesis

The AgNP using FRW extract were synthesized as reported earlier[7] with slight modifications. 50ml of extract was placed in a burette and added dropwise to 100 ml of 0.1 M AgNO₃ aqueous solution. The color of the AgNO₃ solution started to change from colorless to yellow and finally dark brown after the addition of extract. This shows the formation of AgNPs with the addition of FRW extract. The SPR for the solution was recorded



using a UV-vis spectrophotometer. To optimize the reaction conditions following the procedure was followed.

2.3.2. Synthesis of FRW-AgNPs using optimized conditions.

As explained in the later section of the results and discussion the optimum condition (20 ml (0.01 M AgNO₃): 15 ml, (1% Extract) ratio, 50°C temperature, and 30 min time) was followed to produce AgNP efficiently. And the FRW-AgNPs

were separated from the colloidal suspension by filtration process. The thus separated FRW-AgNPs were washed with water (three times) followed by ethanol to remove unreacted AgNO₃ and alcohol-soluble phytoconstituents adhering to the particles. After drying in a hot air oven at 50°C for 2–3 hrs, the particles were powdered in a mortar and transferred into light-resistant containers for further characterization. The process was repeated until enough AgNPs were obtained for characterization.

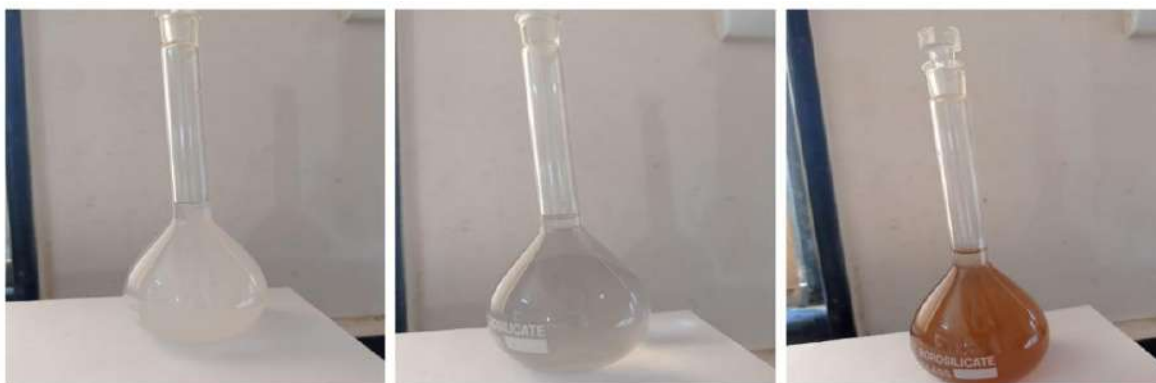


Fig.1 (a) Solution of fermented rice water extract; (b) Aq. solution of silver nitrate; (c) Mixture of fermented rice water extract and silver nitrate



Fig.2 Color Change of Silver Nanoparticle Solution from Light Brown to Dark Brown

2.4. Silver nanoparticles characterization

To study the AgNPs formation, the dried and powdered FRW-AgNPs were dispersed in DI water and sonicated for dispersion, and then scanned by using the UV visible spectrophotometer (Shimadzu-1240, Tokyo, Japan) at the wavelength range of 300–800 nm.

Perkin Elmer21 FT-IR spectrometer was used to obtain FTIR spectra of the dried extract and FRW -AgNPs using the KBr pellet technique. The surface morphology and shape of FRW -AgNPs were assessed with the Field emission scanning electron microscope (FE-SEM, JEOL Corp.,JSM6700F). The FRW-AgNPs sample was

placed on a double-sided carbon adhesive tape on the SEM aluminum stub and coated with gold plasma spray (Sputter Coater-108 Auto, Cressington). The characterization of the crystal structure of the FRW-AgNPs powder was analyzed by x-ray diffractometer (XRD) (Rigaku Miniflex diffractometer) operating with Cu-K α radiation ($k \frac{1}{4} 1.54 \text{ \AA}$) Samples were scanned at the 2 θ range of 10–80 with a 0.1° step size and a step time of 1 s. The particle size of FRW-AgNPs was calculated using the Scherer's equation (Equation 1) [7,8]

$$D = \frac{K\lambda}{\beta^{1/2} \cos\theta} \quad (1)$$

2.5. Antibacterial studies

To test the antibacterial activity of FRW-AgNPs the agar well diffusion procedure was used according to the previous method with slight modifications[9]. The Gram-positive *Enterococcus faecalis*, *B. subtilis* *S. aureus* and Gram-negative *P. aeruginosa*, *E. coli*, *C. tropicalis* pathogenic bacteria were selected for the antibacterial activity of AgNPs. The volume of 30–35 ml of agar media nutrient was transferred to sterile Petri plates. After the medium solidified, 100 ml of the working stock culture (1×10^8 cells/ml) was spread with a sterile cotton swab and then holes were made on the agar with stainless steel cylinders. Various concentrations of AgNPs (10 to 25 mg/ml) were introduced into the Petri dish wells and kept in a refrigerator for 30 min for effective diffusion. The plates were then incubated at 37°C for 24 hrs in a BOD incubator. [10]

2.6. DPPH free radical scavenging assay

The DPPH free radical scavenging assay was performed according to the previous method [11] 5 μ l of different stock of the test compound (As per mention in excel sheet) was added to 0.1 ml of 0.1mM DPPH solution in a 96 well plate. The

reaction was set in triplicate form and duplicates of blank was prepared containing 0.2 ml DMSO/Methanol and 5 μ l compound of different concentrations (As per mention in excel sheet). The wells without treatment were considered as control and wells without reagent (DPPH) were considered as Blank. The plate was incubated for 30 min in dark. At the end of the incubation, the decolorization was read 517 nm using a micro plate reader (iMark, BioRad). Reaction mixture containing 20 μ l of deionized water was served as Control. The scavenging activity was presented as '% inhibition' with respect to control. IC_{50} was calculated using Software Graph Pad Prism 6. Graph was prepared between X axis (Sample Concentration) Vs. Y axis (% inhibition wrt control).[12,13] The DPPH methanolic solution without sample served as control and the percentage inhibition was determined by the following formula (Equation 2).

$$\text{Percentage DPPH inhibition (\%)} = \frac{(\text{control abs}) - (\text{sample abs})}{(\text{control abs})} \quad (2)$$

2.9. Statistical evaluation

The DPPH assay and Mushroom tyrosinase activity were statistically evaluated in terms of the mean and its standard deviation (mean \pm SD) and student's t-test from the results of triplicate experiments.[14] The ic_{50} values were determined with Sigma plot and Origin Pro data analysis and graph software (version 8). Results were considered significant at a 95% confidence level when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectral characteristics of FRW-AgNP

Synthesis of AgNPs from silver nitrate was carried out via an eco-friendly rapid green synthetic route



using 1% fermented rice water (FRW) extract. Successful AgNPs formation was confirmed by changing the transparent color AgNO₃ to a dark brown solution (Figure 1) i.e., the hue of the solution changes from initially transparent to watery yellow then to light yellow and gradually turns dark brown. In contrast, with the reference control, the pure AgNO₃ solution did not show any color change, thus suggesting that the by the size of nanoparticles increasing with time. This

appearance was observed for up to 90 min after no change in absorbance was observed which means there were no remaining Ag⁺ solutions to reduce. It can be inferred that shifts toward blue or red in the λ_{max} of the SPR peaks may be associated with the production of AgNPs with different shapes, sizes, or solvent dependencies of AgNPs. Whereas at the end of the reaction (3 h), there was a slightly more shift in λ_{max} (390 nm) shows in figure No.3.[15]

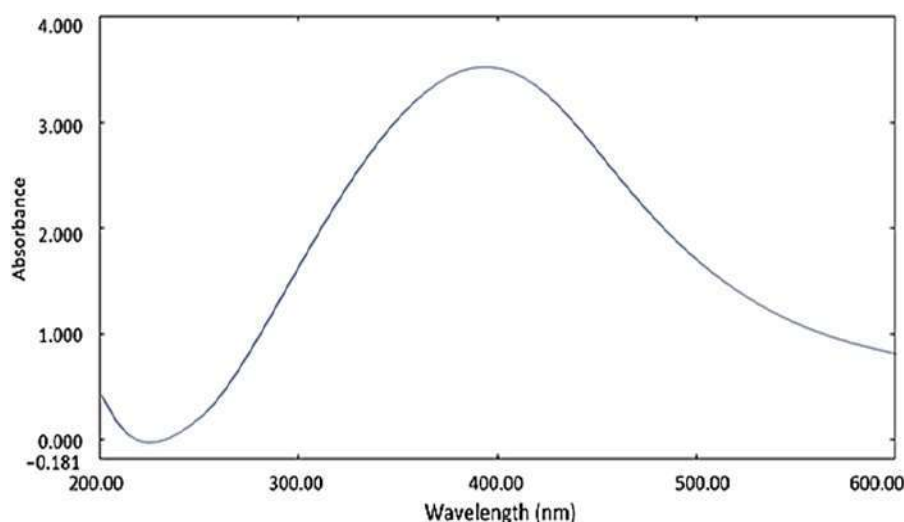


Figure No.3 UV-Spectroscopy of FRW-AgNPs

7.2. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR measurements were performed to identify the main functional groups in the FRW extract and their possible contribution to the synthesis and stabilization of silver nanoparticles [16]. The spectrum of the FRW extract powder is presented in Figure 4. The bands appearing at around 1752 cm⁻¹ were attributed to the C=O stretching vibration of carboxylic acid or ester and the band that appears near 1116 cm⁻¹ was given the responsibility to the C-O-C, the bands at 1292 and 1299 cm⁻¹ were assigned to stretching vibration of nitro compounds, C-N amine bond, respectively.

In addition, the broad band from 3000 to 3500 cm⁻¹ was detected because of the presence of -OH group of phenolics and the NH group of the amines. In the case of the FTIR spectrum obtained for the FRW-AgNPs (Figure 5,6), all the major vibrational bands for the major metabolites were identified from the FTIR spectrum of the FRW - AgNP's as like spectrum of FRW extract, but their peak intensity was lower than that of the original extract, which are believed to be accountable for the decrease of Ag⁺ to Ag⁰ nanoparticles and as stabilizers of the formed nanoparticles by deposition on the particles. These peaks are observed in graphs and specific peaks are mentioned in peak tables. [17]

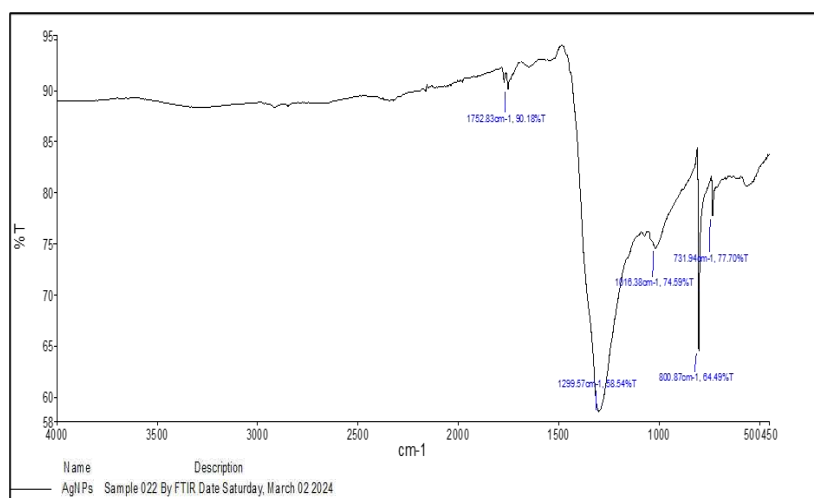


Figure No.4 FTIR spectra of FRW-AgNPs

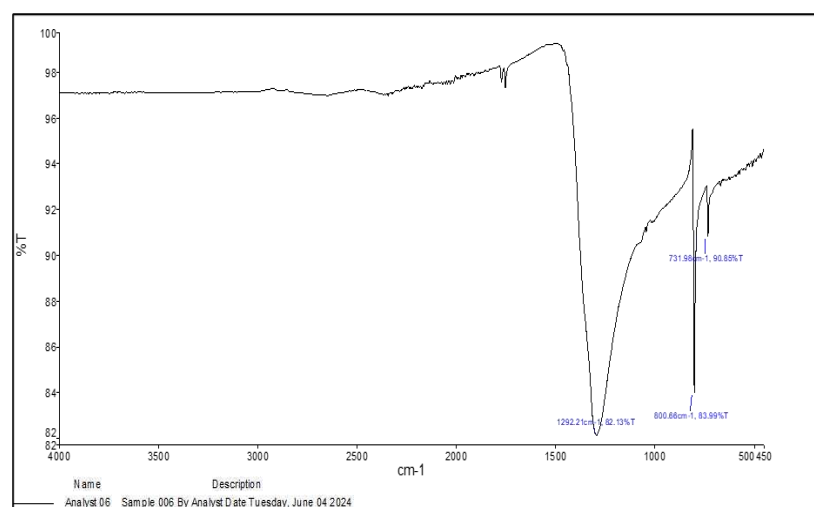


Figure No.5 FTIR spectra of AgNO₃

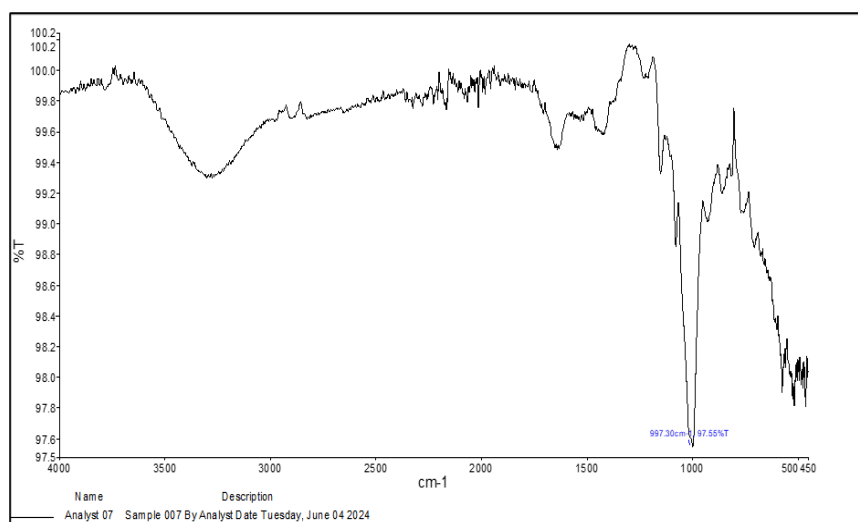


Figure No.6 FTIR spectra of FRW

7.3. X-ray diffraction (XRD)

XRD is an analytical technique broadly used to observe the structure of crystalline metallic nanoparticles by penetration of X-rays deeply into the material. The resulting diffraction pattern confirms the formation of nanoparticles with crystalline structure. To calculate the particle size from the XRD data, the Debye–Scherrer equation is applied by determining the width of the Bragg reflection law according to the equation: $d = K\lambda/\beta \cos \theta$, where d is the particle size (nm), K represents the Scherrer constant, and λ denotes the wavelength of the X-ray. β is the full width half maximum and θ is the diffraction angle (half of Bragg angle) that corresponds to the lattice plane. As a result, the structural characteristics of different materials, including biomolecules, polymers, glasses, and superconductors, can be

analyzed through XRD. In addition, XRD is a potent method for the study of nanomaterials. The XRD pattern clearly indicates that the silver nanoparticles synthesized in this study are of crystalline structure. In addition, some unassigned peaks it was also observed, suggesting that the crystallization of the bioorganic phase took place on the surface of the nanoparticles. [18] The crystalline nature of synthesized FRW-AgNPs was analyzed by acquiring an XRD pattern. and were indexed with corresponding lattice plane values of (111), (200), (220), and (311). (Figure 7). The appearance of sharp peaks in the XRD of green synthesized FRW-AgNPs showed that the fermented rice water extract was more efficient in stabilizing the formed nanoparticles. Other co-existing low-intensity peaks were caused by adhering to organic impurities in the sample.[19]

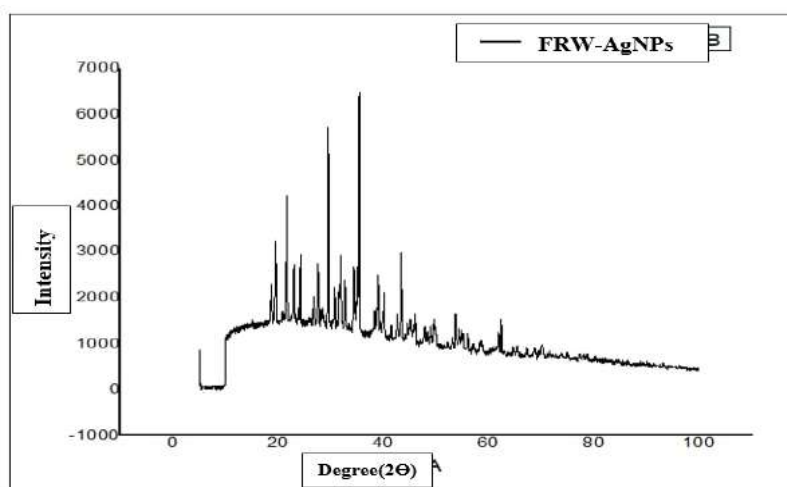


Figure no. 7 X-ray diffraction of silver nanoparticles

7.4. Field Emission Scanning Electron Microscopy (SEM)

The FE-SEM images of the prepared FRW-AgNPs were portrayed in Figures 8. The images clearly reflect that the synthesized nanoparticles were

spherical in shape. Further, the particle size measurements with SEM image-J software was resulted that the average size of 200 particles measured being 26 nm which is concordant with that of particle size measured with surface Plasmon resonance peak at 390 nm.[20]

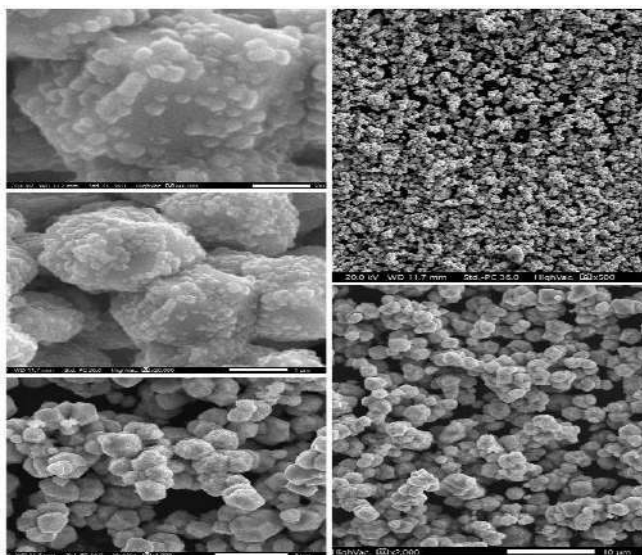


Fig.No.8 Scanning Electron Microscopy of silver nanoparticles

7.5. Antimicrobial activity of silver nanoparticles synthesized by using fermented rice water extract

The nanoparticles under 100 nm size are of prime choice in controlling the eukaryotic microbes, such as bacteria (Gram (+ve) and Gram (-ve)), fungi, and viruses, because of their greter surface-to-volume ratio. Figure 9 portrayed the results of anti-microbial activity exhibited by the FRW–AgNPs. It was observed that the FRW–AgNPs are more effective in inhibiting Gram-negative pathogenic bacteria compared to Gram-positive bacteria. The inhibition zones created by FRW–AgNPs on *Enterococcus faecalis*, *Bacillus subtilis*, *Staphylococcus aureus* bacteria were higher than gram (-ve) *P. aeruginosa*, *E. coli*, *C. tropicalis*. The exact mechanism of the AgNPs antimicrobial property against the microbes has yet to be revealed.[10] However, there has been extensive research devoted to these concerns to explicate their mechanism of action; in this regard, three well-defined plausible mechanisms have so far

been proposed are as follows: After the addition of AgNPs they were start adhesion over the cytoplasmic membrane of the bacteria then this will followed by penetration in to the bacterial cytoplasmic membrane through the pores induced by them, through the pores formed by the entry of nanoparticle allow inner cellular to be leaked out these will further leads to cell death by rupturing of the cell membrane. By their oxidizing potential entered AgNPs act on the surface of proteins found in the plasma membrane and causes a cellular homeostasis which is considered as one of primary mechanism of AgNP antibacterial activity along with diminished permeability and cell respiration by cell surface adhesion. [21] Gram +ve bacteria: *Enterococcus faecalis*, *B. subtilis*, *S. aureus*; Gram –ve bacteria: *P. aeruginosa*, *E. coli*, *C. tropicalis*; Antifungal Strain: *C. parapsilosis*. Based on the results obtained from the study, some of the effects of FRW-AgNP were observed up to 1 mg of the compound which was different with respect to positive control and are mentuin in tables 1 and 2.

Table No.1: Max. Zone Inhibition of bacteria *B. subtilis*, *E. faecalis*, *S. aureus*, *S. marcescens*, *P. aeruginosa*, *E. Coli*

Sr. No	Sample	Max. Zone Inhibition of bacteria(mm)	

		B. subtilis	E. faecalis	S. aureus	S. marcescens	P. aeruginosa	E. coli	Amount Loaded (µg)
1	Ciprofloxacin	27	27.5	25.66	22	23	19	10
2	FRW-AgNP	16	18	18	24	17	15	1000

Table No.2: Max. Zone Inhibition of bacteria *C. tropicalis*, *C. Parapsilosis*

Sr. No	Sample	Max. Zone Inhibition of bacteria(mm)		Amount Loaded (µg)
		<i>C. tropicalis</i>	<i>C. parapsilosis</i>	
1	Amphotericin B	15.66	20.66	50
2	FRW-AgNP	10.33	17.66	1000

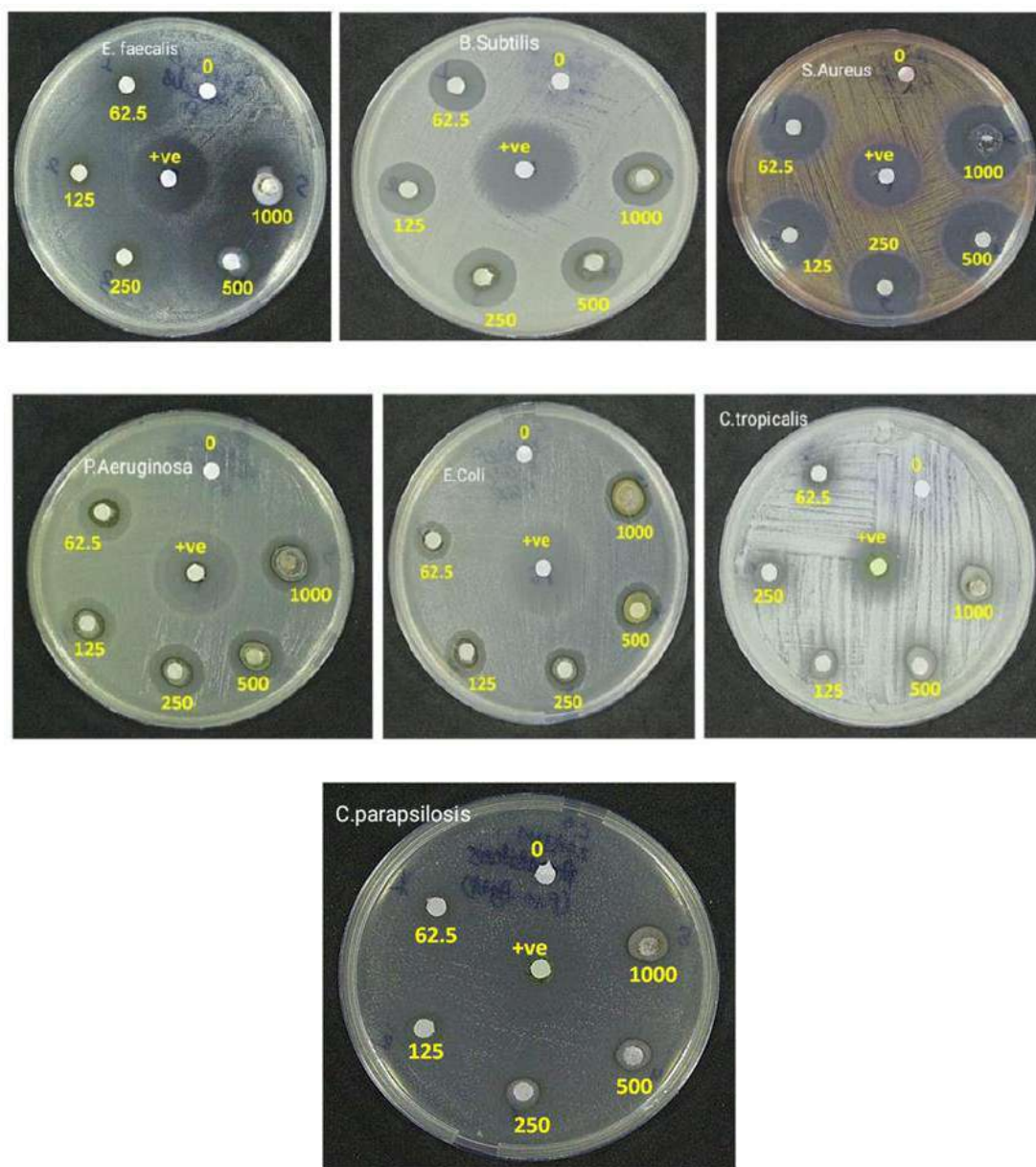


Figure No.9 Antimicrobial activity of FRW-AgNPs against Gram +ve bacteria *Enterococcus faecalis*(a), *B. subtilis*(b), *S. aureus*(c); Gram –ve bacteria *P. aeruginosa*(d), *E. coli*(e), *C. tropicalis*(f); Antifungal Strain - *C. parapsilosis*(g)

7.6. Antioxidant activity- DPPH scavenging assay

Green-synthesized metal and metal oxide nanoparticles have been intensively reported recently for their efficient antioxidant capabilities.[22] In this research, the antioxidant properties of FRW-AgNPs, and its antioxidant activity, were assessed against DPPH, and compared with those of ascorbic acid (AA), a natural antioxidant. Fig. 10 (a) presents the results of the assay. The results of the DPPH scavenging assays demonstrated that the synthesized FRW-AgNP exhibited notable antioxidant activity. (Fig. 10(b)). The ability to scavenge radicals of the

FRW-AgNP increased with increase in concentration from (1.0 to 250) $\mu\text{g/mL}$, and the percentage of inhibition ranged ((10.8 \pm 0.027) to 75.2 \pm 0.057)) % Fig. 14 (a) The AgNPs exhibited the highest percentage of inhibition of (75.2 \pm 0.057) %, with the concentration of 250 $\mu\text{g/mL}$. The IC_{50} value (AgNP required to neutralize 50 % of DPPH). Based on the The findings derived from the experimental work, Antioxidant activity (DPPH Assay) was estimated in samples and 50% inhibitory concentration (IC_{50}) were mentioned in table 8. Sample FRW-AgNP was determined to be very less active (Very Low DPPH Scavenging) as compared to standard Ascorbic acid.

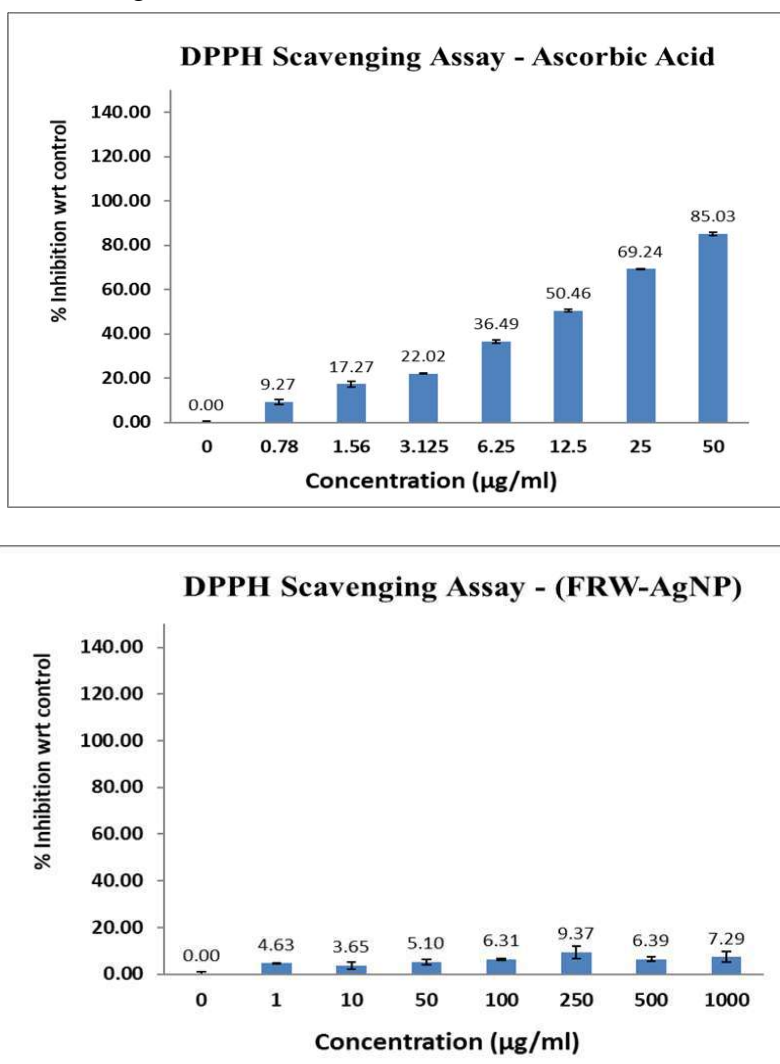


Figure No.10 (a) DPPH antioxidant radical scavenging test of ascorbic acid, (b) DPPH antioxidant radical scavenging test of FRW-AgNPs

Table No.8: Standard Deviation of DPPH Scavenging Assay

Sample code	IC ₅₀ value (µg/ml) (Mean± SEM)
Ascorbic Acid	10.8 ± 0.027
FRW- AgNP	75.2 ± 0.057

CONCLUSION

Synthesis of the silver nanoparticles using bio-inspired green chemistry has been found to be an energy-efficient and cost effective, environmentally friendly, and renewable method that is more needed in the latest environmental scenario. My present work emphasizes the simplified and faster synthesis of the FRW-AgNPs using the fermented rice water extract. The resulting FRW-AgNPs have mean particle size of 26 nm. The in-vitro biological activity results of synthesized AgNPs proved their potential to act as an antimicrobial, free radical scavenger. Based on this, I conclude that the FRW-AgNPs synthesized in this work system with an excellent antimicrobial, free scavenging (Antioxidant activity). The route of synthesis of AgNPs is green and renewable as well. This study demonstrates the significant antimicrobial potential of fermented rice water (FRW) and fermented rice water silver nanoparticles (FRW-AgNPs) against clinical isolates of *Enterococcus faecalis*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *C. tropicalis* and Antifungal Strain (*C. parapsilosis*). FRW- AgNPs exhibit enhanced antimicrobial effects compared to FRW alone.

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