Microwave Assisted Green Synthesis of Silver Nanoparticles with Leaf of *Ficus racemosa* and its *in vitro* Antibacterial Analysis and Dye Catalytic Activity

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Abstract

This study reports an investigation of the microwave-assisted synthesis of silver nanoparticles (AgNPs) using an extract of Ficus racemosa leaves. The formation of AgNPs was established by various techniques such as UV-Visible Spectroscopy (UV-Vis) analysis, Scanning electron microscopy (SEM) for shape and size analysis and Fourier Transformed Infrared spectroscopy (FTIR) for phytochemical analysis involved in reduction of silver ions. Further antibacterial activity was tested against P. aeruginosa, B. cereus, C. jejuni, B. subtilis, L. Monocytogenes, and C. perfringenes.

The results indicated the rapid formation of AgNPs during microwave irradiation with improved properties to those obtained by the heating method. The use of microwaves resulted in the smaller size of particles approximately 30 to 60nm. Prepared nanoparticles demonstrated antibacterial property against some bacterial strain and dye degradation.

Keywords: Green Synthesis, UV-Vis, FTIR, SEM, Antimicrobial activity, AgNPs, dye degradation.

Introduction

Synthesis of nanoparticles using phytochemicals is most trending in the present scenario due to their unique properties. Nanoparticles can be synthesized using the various physiochemical methods like gamma-ray irradiation [6], micro-emulsion[31], microwave irradiation[16], laser ablation[1], electrochemical reduction[22], autoclaving[30], chemical reduction[15] and photochemical reduction[3]. Among these, green synthesis is a better technique for synthesizing the nanoparticles because it is cost-effective and environment-friendly[5].

Metallic nanoparticles are frequently used in medical science. Among all metals, silver has a strong antimicrobial activity due to its toxicity against most microorganisms like bacteria, fungi, and viruses[26]. Extracts of plants and its derivative extracts have been used as a reducing agent in the green synthesis of silver nanoparticles (AgNPs). Metabolites present in the plants like alkaloids, phenolic acids, terpenoids, sugars, polyphenols, and proteins play an important role in the bio-reduction of silver ions to Ag NPs and stabilize the synthesized nanoparticles[32].

Many researchers have worked on the green synthesis of AgNPs using various plant extracts including *Hibiscus rosasinensis, Azadirachta indica,* black tea, *Emblica officinalis, Cinnamomum camphora, Cinnamomum zeylanicum, Parthenium Camellia sinensis, Magnolia kobus, Diopyros kaki, Geranium,* natural rubber, Alfalfa, aloe[21], *Morganella psychrotolerans* leave extracts[19], *Pelargonium zonali* leaf extract[18] and *Aloe vera*[2].

Textile industries and others such as paper, pharmaceutical and food industries etc. widely used organic dyes or pigments to add colors to their products[29]. They use excessive amounts of organic dyes and water leading to water pollution and water pollution which is a major threat to environment and animal health including humans[20] Moreover, some dyes may be carcinogenic and have high toxicity which endangers the life of animals[10,23]. For that reason, the removal of such dyes from the water that has been already polluted by industrial effluents is environmentally important[12].

For the remediation of pollutant dyes in water, different traditional methods are used such as chemical treatment, physical, biological processes. Adsorption and degradation methods have advantages among them because these are cost-effective, and they do not release toxic substances[11]. The various number of nanoparticles such as Au[4], ZnO[14], Pd[25] and Pt[9] have been extensively exploited for the degradation of dye materials.

In the present study, synthesis of AgNPs using *F. racemosa* leaf extract in aqueous form by microwave exposure has been reported. The antimicrobial activity of AgNPs synthesized by *Ficus racemosa* (*F. racemose*) has been evaluated. *F. racemosa* a tree, belonging to the family of the Moraceae is commonly known as 'Goolar'.

Silver metal has been chosen because nanosized silver shows efficient toxicity to micro-organisms and lesser toxicity with minimal side effects in mammalian cells[27]. Here, a rapid and simple synthesis of AgNPs by reduction of $AgNO_3$ with leaf extract of *F. racemosa* under microwave exposure has been

performed. We have evaluated the effects of (i) leaf extract quantity in synthesis of AgNPsand (ii) antibacterial activity.

Material and Methods

Materials: Absolute Ethyl alcohol, $AgNO_3$ powder, Mueller-Hinton agar (MHA) powder were procured from Merck. The fresh leaves of the medicinal plant, *F. racemosa* were procured from the garden of Indian Institute of Information Technology, Jhalwa, Allahabad, India for the synthesis of silver nanoparticles.

Synthesis: The fresh leaves of *F. racemosa* were collected and cleaned in the flow of faucet water and dried in air to remove moisture and granulated into fine powder. Leaves powder (2.0 gm) were soaked in 30 ml double distilled water over night. It was then filtered through Whatmann filter paper to get the extract solution. Powder of Silver Nitrate ($AgNO_3$) was taken and mixed in measured amounts of double distilled water to make aqueous 0.02M solution and the prepared stock solution of $AgNO_3$ was kept at 4^{o}C for further use.

One ml of plant extract was added in 20 ml of 0.02M $AgNO_3$ solution. Mixture was kept in the microwave oven for 30 seconds. The color of mixture changed to yellow which indicates the synthesis of AgNPs. After synthesis, the sample was centrifuged at 9000rpm for 10 minutes to collect pellets. Then it was dried to get the powder form for further process.

Characterization of synthesized silver nanoparticles: Production of silver nanoparticles was determined by UV-Vis spectroscopy and absorption spectra were taken at 300-600nm, Double distilled water was used as blank. Morphological study and size of AgNPs were determined by Scanning electron microscopy (SEM) (Model- JSM-6490LV). The preparation sample was required due to the nonconductive nature of synthesized AgNPs. Sputter coating was performed to make it conductive. EDX was also performed to elemental analysis.

The graph generated from X-Ray diffraction (XRD) of nanoparticle was shown in which range of 2θ value was taken 20 ^{o} - 80 ^{o} with 0.02 step using Kα1 at 1.540 and Kα2 at 1.544 with scan mode of 2θ/θ. The FTIR of dried AgNPs was characterized using Nicolet 6700 FTIR instrument within the range of 27000 – 15 cm^{-1} using Potassium Bromide pellets process (NicoletTM6700, Make- Thermo Scientific, USA).

Antibacterial: Antibacterial activity of synthesized AgNPs was investigated on Muller Hinton agar plates by using Agar well diffusion assay[17]. In sterilized Petri dishes, Muller Hinton agar was poured and allowed to solidify. Overnight broth culture of the test bacteria was swabbed on the plates uniformly with the help of sterilized cotton swabs. With sterile stainless-steel cork borer, 5mm diameter wells were cut on the inoculated Muller Hinton agar plates. 0.1ml of the AgNPs solution was filled into well and plates were then incubated at 37°C for 24h. The antibacterial activity of AgNPs was confirmed by the presence of zone of inhibition millimeter.

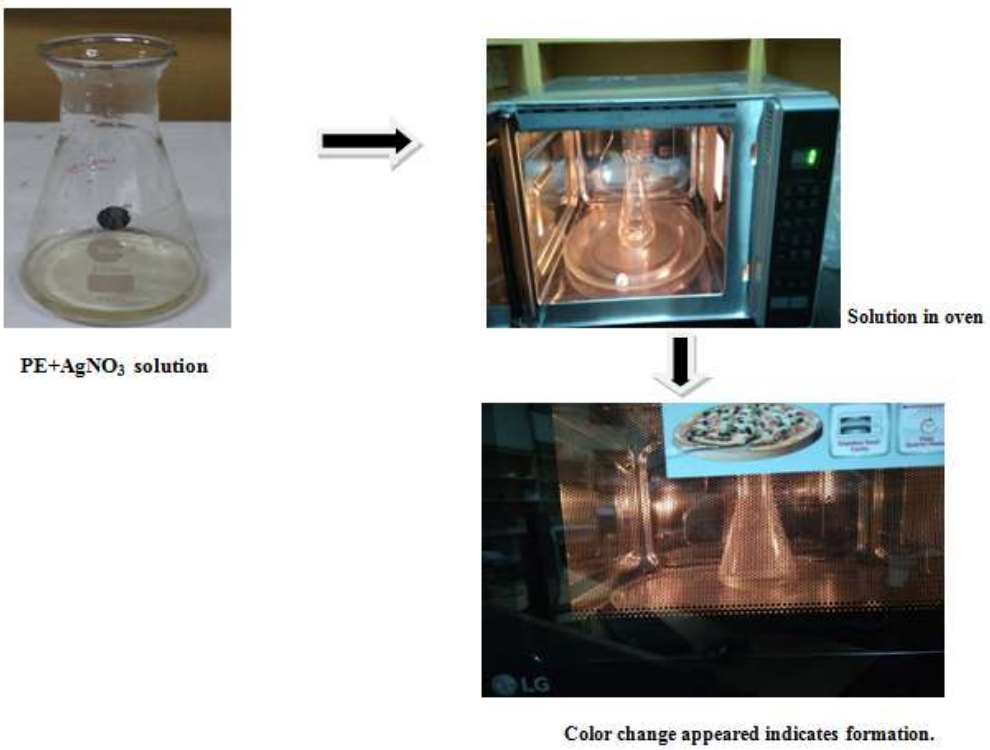


Figure 1: Microwave exposure to silver nitrate and leaf extract mixture for synthesis of silver nanoparticles

Minimum Inhibitory Concentration: The minimum inhibitory concentration of synthesized AgNPs was determined by the broth dilution technique[7]. To achieve different concentrations, AgNPs were serially diluted in nutrient broth medium. Bacterial culture was then added to the different tubes. The tubes were finally incubated at 37°C for 24h and observed for the growth of the bacteria. The least concentration of each AgNPs showing a clear inhibition was taken as the MIC level. The microorganisms used to study the inhibitory effect of silver nanoparticles were; *P. aeruginosa*, *B. cereus*, *C. jejuni*, *B. subtilis*, *L. monocytogenes*, and *C. perfringenes*.

Dyes Degradation: Aqueous solutions of MB, BB, and MO with a concentration of 0.01% and 0.001% of each were prepared and mixed with nanoparticles. After adding 2 mg of the AgNPs in the solution of dyes, the mixtures were examined by taking absorbance by UV-Vis spectrophotometer at a different time interval to determine the concentration of MB, BB, and MO during degradation.

Results and Discussion

Biosynthesis of AgNPs using the leaf extract of *F. racemosa* as a reducing agent is more productive in comparison to other bio-agents[17,18,28]. Hence, *F. racemosa* is exploited for the biosynthesis of AgNPs.

UV-visible spectroscopy: Reaction of silver nitrate and leaf extract of *F. racemosa* occurs in one step; synthesis of silver nanoparticles was indicated by the color change during reaction which is reddish-brown which was further analyzed by UV-Visible spectroscopy. The result showed that absorption bands at the wavelength of 390-400nm in UV-VIS range at a different time interval were observed using UV-VIS Spectrophotometer. On increasing the reaction time, increase in peak intensity denotes the increase in the concentration of silver nanoparticles which are stable due to the coating nature of leaf extract ingredients.

XRD analysis: The graph generated from X-Ray diffraction of nanoparticle was shown in which range of 2θ value was taken 20° - 80° with 0.02 step using Kα1 at 1.540 and Kα2 at 1.544 with scan mode of 2θ/θ. The crystalline size of nanoparticles is calculated using Scherrer's formula i.e. $D = K\lambda/\beta\cos\theta$, where K is Scherrer's constant = 0.94, λ is wavelength=0.154nm and β is FWHM (Full Width at Half Maximum) of peaks[8]. The crystal size of the synthesized AgNPs is 50.03nm.

The planes in the fcc (Face centered cubic) structure of AgNPs observed 2θ values of diffraction at 18°, 20°, 22°, 30°, 33°, 35°, 40°, 42°, 45°, 55° (figure 3) which corresponds to the peaks shown in diffractogram. Some small peaks were also found at 45° and 50° due to the capping of silver. Small peaks were found due to organic phytoconstituents involved in the capping of AgNPs. Sharp peaks with high intensity indicate that AgNPs are in its crystalline phase.

SEM analysis: Micrograph of SEM shows the AgNPs in average particle ranges between 1-100 nm and depicted the spherical morphology of AgNPs and sizes of synthesized silver nanoparticles range between 30- 60 nm which are within the given standard range displaying the formation of silver nanoparticles.

EDX microanalysis: After the reaction, the yield of AgNPs by % weight is 79.62 as major component present in the powdered sample of AgNPs along with chlorine (11.9%) and carbon (8.46%) which are due to bio-molecules of plant extracts reacting with the silver revealing the reduction process of silver ion to metallic silver. The spectrum of Energy Dispersive X-Ray Analysis (EDX) exposes powerful signal in the region of powdered silver and declares the formation of silver nanoparticles. AgNPs shows an absorption peak at approximately 3keV because of SPR. The Y-axis shows the total number of X-rays while the X-axis shows energy (KeV).

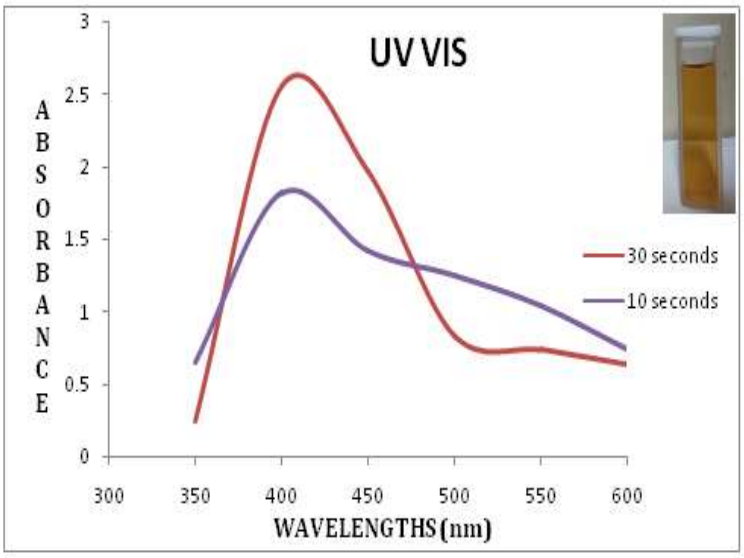


Figure 2: UV-Vis absorption of synthesized silver nanoparticles showing at 430nm of wavelength

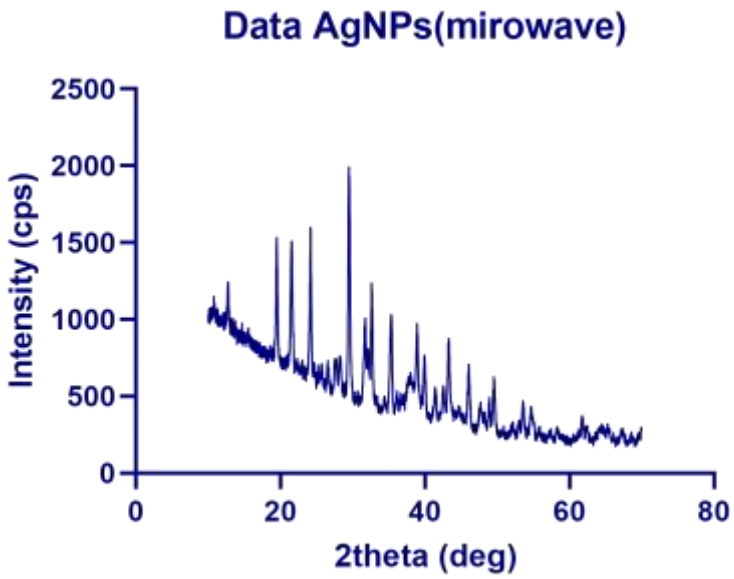
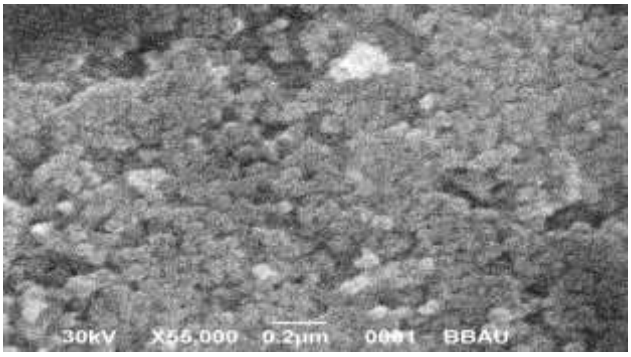
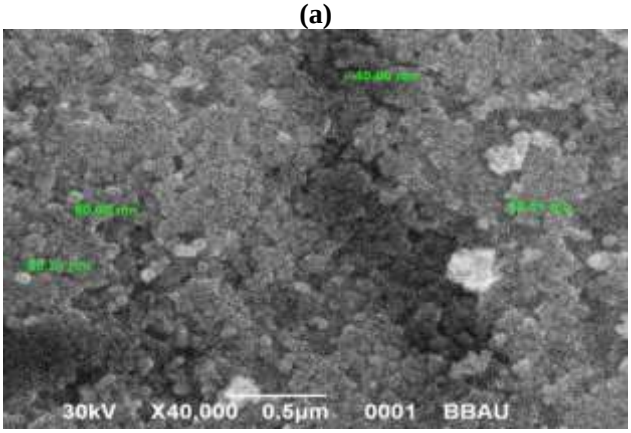


Fig. 3: XRD peaks of synthesized AgNPs showing its crystalline nature



(a)



(b)

Figure 4: Shape and Sizes of Silver nanoparticles at different magnification powers

FTIR analysis: Plant compounds and their functional groups which are involved in the synthesis of AgNPs have been characterized by FTIR (Fourier Transformed Infrared spectroscopy). The characterization was done to identify several bio-molecules present in leaf extract of *F. racemosa*. So, a dried sample of AgNPs was analyzed by FTIR in which the peaks were obtained. The extreme peak at 3426.1cm^{-1} is hydrogen-bonded phenol which indicates the presence of a phenolic functional group, responsible for reducing silver ion. The peak at wavenumber 2911.9 cm^{-1} indicates C-H stretch and also revealing the presence of carboxylic acid.

The presence of alkynes is revealed by the peak 2152.1cm^{-1}. The peak at 1635.4 cm^{-1} indicates the presence of the C=C stretch of alkenes. The peak at 1643.4 and 1559.3 cm^{-1} indicates the presence of stretch aromatic of C=C which reveals the capping agents of the aromatic group. The peak at 1384.4 cm^{-1} indicates a stretch of alkanes.

The peak at 1236.1 cm^{-1} shows the presence of a C-O stretch of ether, alcohol linkages which confirm the presence of flavonoids in plant extract used as reducing agents and are acting as capping agents on the surface of nanoparticles by adsorption and C- N amines as a stretch of protein. The peak at 1070.7 cm^{-1} indicates the presence of O-H stretch[13]. So, a detailed study of FTIR peaks of absorbance and transmittance revealed interaction of silver ion and plant extract of *F. racemos*a Linn. leaves.

***In Vitro* Anti-Bacterial activity analysis:** Synthesized AgNPs are found active against the tested bacterial strains. Nanoparticles showed activity against *P. aeruginosa* and *C. perfringenes*, suggesting its broad-spectrum activity against both gram positive as well as gram negative bacteria. Other bacterial cultures were found resistant against synthesized AgNPs as no zone of inhibition was recorded against them. ZOI of synthesized AgNPs is better than known standard antibiotics (Colistin and Streptomycin).

Element	Weight%	Atomic%
C K	8.46	39.62
Cl K	11.91	18.89
Ag L	79.62	41.50
Totals	100.00	

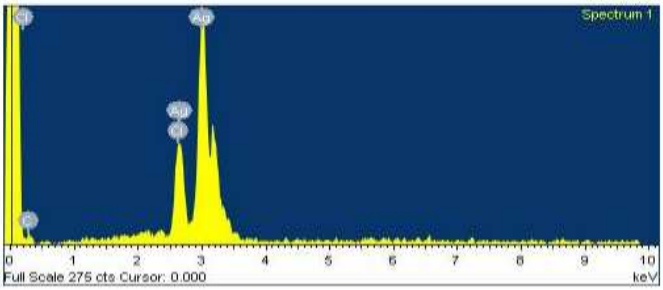
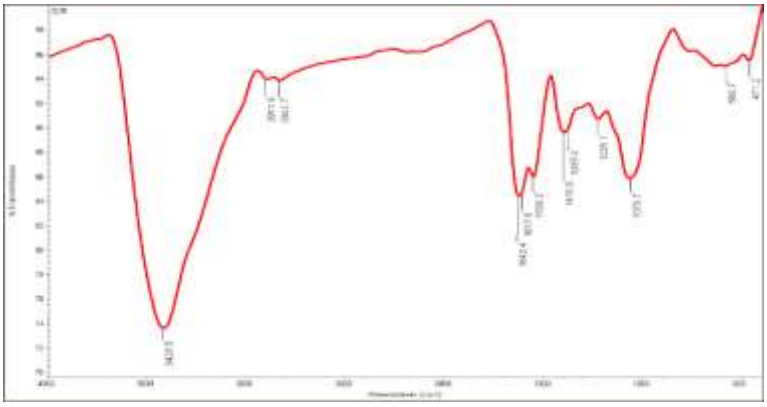
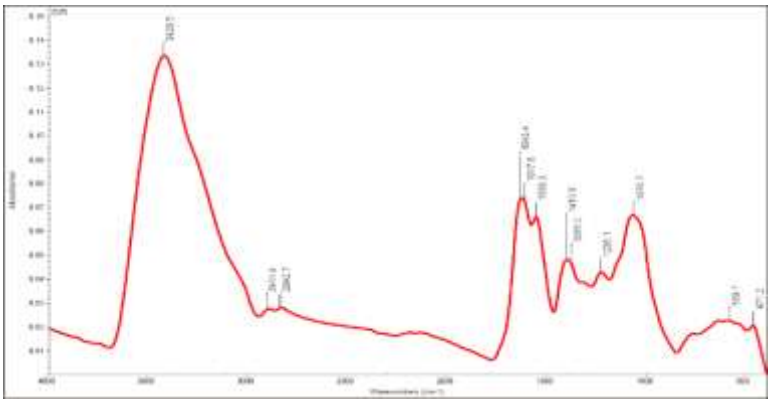


Figure 5: EDX result of silver nanoparticles showing amount silver in synthesise nanoparticles



(a)



(b)

Figure 6: (a) Transmittance and (b) Absorbance peaks of FTIR of silver nanoparticles

Table 1
Antibacterial activity of AgNPs against selected bacteria and their Minimum inhibitory concentration (MIC)-

S.N.	Organism	Compound				Antibiotics	
		AgNPs		Plant Etract		Colistin	Streptomycin
		ZOI(mm)	MIC(µg/ml)	ZOI(mm)	MIC(µg/ml)	ZOI(mm)	ZOI(mm)
1.	*P.aeruginosa*	35	8	-	-	17	21
2.	*B. cereus*	-	-	-	-	0	29
3.	*C. jejuni*	-	-	-	-	0	29
4.	*B. subtilis*	-	-	-	-	13	26
5.	*L. monocytogenes*	-	-	-	-	0	17
6.	*C. perfringenes*	26	32	-	-	12	31

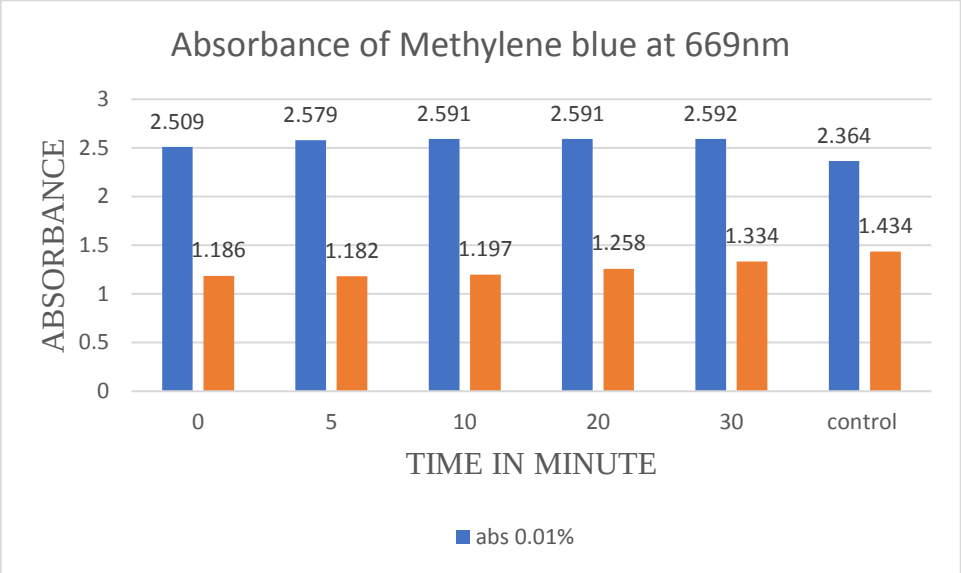


Figure 7: Absorbance of 0.01% and 0.001% aqueous solution of Methylene Blue at 669nm showing increase of absorbance

Minimum inhibitory concentration (MIC) was determined by the broth dilution assay. Organisms showing zone of inhibition in agar well assay were further proceeded to investigate the MIC of compounds. The minimum inhibitory concentration of synthesized silver nanoparticles corresponded to 8µl/ml and 32µl/ml against *P. aeruginosa* and *C. perfringenes* respectively shown in table 1.

Catalytic Degradation of Dyes: Dye MB degradation was investigated by the synthesized AgNPs at different time of intervals. The absorbance peak of MB was taken at 669nm. As the incubation time increases of AgNPs and MB aqueous mixture, the absorbance of MB dye increases. 2.364 and 1.434 are the absorbances of 0.01% and 0.001% solution of MB at 669nm respectively. After adding AgNPs, the absorbance was taken from 0 to 30 minutes which shows the increase of absorbance significantly which indicates the reduction of MB with an increase in time.

The absorbance MO dye solution of 0.01% and 0.001% has 2.889 and 0.382 at 464nm respectively. The solution of MO dye was treated with the AgNPs and absorbance was taken from 0 to 30 minutes. An increase in the absorbance of MO with respect to time has confirmed the degradation of MO and after maximum degradation, the rate of increase in the absorbance halted.

The solutions of BB dye of 0.01% and 0.001% have absorbance 0.735 and 0.068 at 590nm. SRP of the mixture increases from 0.735 and 0.067 to 0.992 and 0.160 respectively at zero minutes after treating with AgNPs but with the increasing time of incubation, the absorbance peak decreases which indicates the degradation of BB dye[24,28]. Change in the absorbance of dyes shows the catalytic nature of synthesized silver nanoparticles. Hence it degraded to the MB, MO and BB dyes.

Conclusion

The green method in the synthesis of metallic nanoparticles has confirmed its significance in industries and health sectors. Despite some restrictions, it has many advantages of synthesizing nanoparticles as compared to physical and chemical methodology because of its eco-friendly nature and cost-effectiveness. In this experiment, the reduction of silver ion at the nanoscale using *F. racemosa* leaves containing phytochemicals through microwave-assisted processes was visualized through its color change and the absorption band

at different time intervals in UV-VIS range of wavelengths was measured through UV-VIS spectrophotometer. The nanoparticle's morphology, size and shape, and presence of silver were confirmed through SEM imaging and EDAX analysis and also the phytonutrient as a reducing agent was confirmed through FTIR analysis. Synthesized silver nanoparticles were found as potent antibacterial and dye degradable bioproduct.

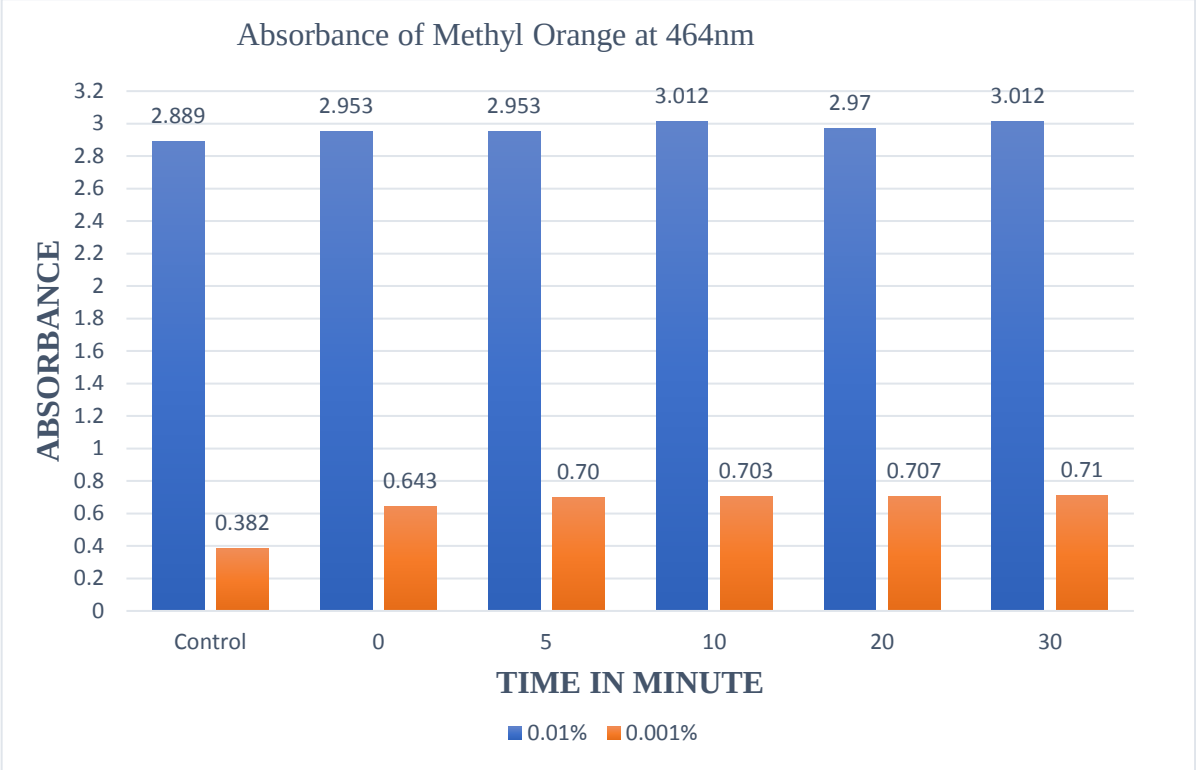


Figure 8: Absorbance of 0.01% and 0.001% aqueous solution Blue of Methyl Orange at 464nm showing increase of absorbance

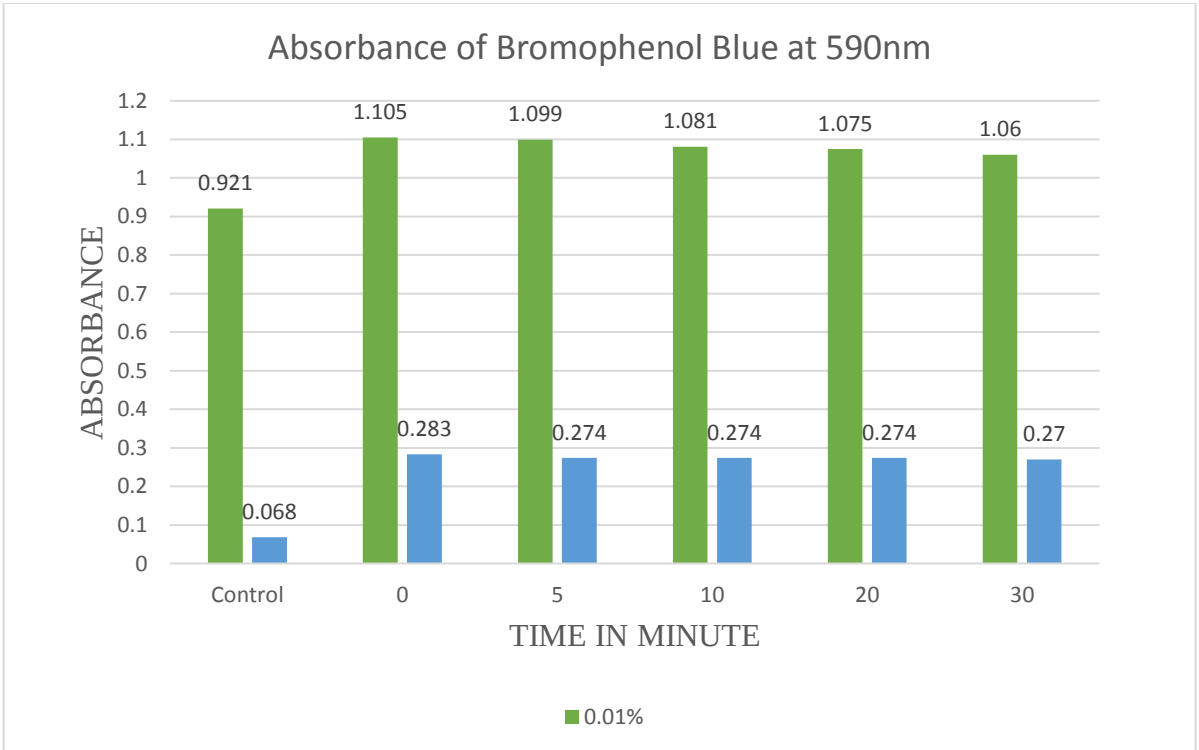


Figure 9: Absorbance of 0.01% and 0.001% aqueous solution of Bromophenol Blue at 590nm showing increase then decrease of absorbance

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