Pharmacological and Photocatalytic Degradation Studies of Synthesized Silver nanoparticles using Triumfetta rotundifolia Plant Extract

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Abstract: The scientific research introduced many new methods for the synthesis of metallic nanoparticles. Green synthesis technique is non-toxic, eco-friendly and low cost method. The green synthesized silver nanoparticles are characterized by using UV–VIS spectroscopy, SEM, XRD, and DLS method. The size and extract dependent catalytic activity of the green synthesized silver nanoparticles is established in the degradation of organic dyes. The Pharmacological studies of synthesized silver nanoparticles are also carried out.

Keywords: Silver nanoparticles, Degradation of dyes.

I. INTRODUCTION

Organic dyes are one of the major groups of pollutants which are widely used in textile, plastic, medicine and many other industries, while the hazardous effects of organic dyes in waste water have been a major concern and now a major threat in the environment due to the substantial pollution problems caused by them. A necessary criterion in the use of these kinds of dyes is that they must be highly accumulated in water and stable in light during washing. The dye accumulation in the water bodies causes eutrophication, reduces the reoxygenation capacity and makes severe damage to the aquatic organisms by hindering the infiltration of sunlight [1]. They must also be resistant to microbial attack which are not readily degradable and are typically not removed from water by wastewater treatment systems and conventional methods like adsorption, ultra filtration, chemical and electrochemical methods [2]. AgNPs can be synthesized using a number of production techniques such as reduction in solution, Chemical and photochemical reaction, electrochemical reaction, sonochemical, microwave and laser technology [3, 4]. These techniques yield low concentration of metallic nanoparticles and which are costly and mainly involve the use of toxic, hazardous chemicals which pose potential environmental and biological risks.

In this study the photo catalytic degradation of methylene blue, congo red and malachite green dye in presence of green synthesized silver nanoparticles using Triumfetta rotundifolia plant extract. This greenish route chosen over common chemical and physical procedures because it is an easy, fast and eco-friendly option and does not involve any costly instrument or hazardous by-product as well [5].

II. MATERIALS AND METHODS

Silver nitrate was purchased from Hi-Media chemicals. Methylene blue, Congo red, Malachite green dyes were received from Sigma Aldrich chemicals. Ultra pure deionised water was used throughout the reactions. Whatmann no.1 filter papers were used. Glass wares used for the complete reactions were washed well and rinsed with double distilled water and dried in hot air oven before use.

Preparation of Plant extract:

5g of the collected plant T. rotundifolia powder was mixed with 100 mL of double distilled water and refluxed for 30 minutes and cooled down to room temperature. The solution was then filtered through whatman No.1 filter paper and the filtrate obtained was stored then at 4°C for future work. Synthesis of silver nanoparticles using plant extract of T. rotundifolia: 5 mL of stock solution of plant extract was slowly added to 20 mL of 1mM solution of silver nitrate solution under room temperature. After the complete addition of plant extract, the colourless solution changed from pale yellow colour, and after 30 minutes colour changed from pale yellow to dark brown colour (Figure 1). The colour change indicates the formation of silver nanoparticles. Then, the solution was centrifuged for 10 min in 10,000 rpm for 15 min, consequently dispersed in double distilled water to remove any heavy biological materials present in synthesized silver nanoparticles. Synthesized silver nanoparticles were dried in an oven at 80ºC for 2 hours

Figure 1 Colour change photography of synthesized silver nanoparticles using T.rotundifolia plant extract

Characterization Techniques:

The synthesized silver nanoparticles were characterized by UV-Visible spectral measurements which were carried out on UV-1800 SHIMADZU UV- spectrophotometer instrument. FTIR, XRD, HR-SEM and antibacterial studies were already reported [6]. The dye degradation studies of the synthesized silver nanoparticles were observed by the above mentioned UV-Vis spectrophotometer. The AFM is an instrument capable of measuring the topography of the synthesized nanoparticles. Electrochemical experiments were carried out by in a three electrode cell. Glassy Carbon electrode (GCE) as working electrode, saturated Ag/AgCl/KCl as a reference electrode and platinum wire as a counter electrode. Thermal decomposition behavior of the synthesized nanoparticles has been studied by using thermal analysis. The DSC-TGA patterns were collected as a function of temperature up to 1000oC under N2 atmosphere. The heating rate was 10oC/minutes in N2. Alumina was used as reference material. The size distribution or average size of the synthesized nanoparticles was determined by dynamic light scattering (DLS) method. The DLS technique uses light to determine the size of particles in a solution.

III. PHOTOCATALYTIC DEGRADATION OF DYES

(i) Evaluation of effect of synthesized silver nanoparticles on the reduction of methylene blue by T. rotundifolia plant extract: 10 mg of methylene blue dye was added to 1000 mL of double distilled water used as stock solution. About 10 mg of green synthesized silver nanoparticles were added to 100 mL of methylene blue dye solution. A control was also maintained without addition of silver nanoparticles. Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30 min to clearly make the equilibrium of the working solution. Afterwards, the dispersion was put under the sunlight and monitored from morning to evening. At specific time intervals, aliquots of 2-3mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at different wavelengths. Concentration of dye during degradation was calculated by the absorbance value at 662 nm [7]. Percentage of dye degradation was estimated by the following formula: % Decolourization =100 × (C0-C) / C0, where C0 is the initial concentration of dye solution, and C is the concentration of dye solution after photocatalytic degradation.

(ii) Evaluation of effect of synthesized silver nanoparticles on the reduction of congo red by T. rotundifolia plant extract: Synthesized silver nanoparticles of T. rotundifolia, is used to decolourize the dye congo red in this experiment. For decolourization study, 1ppm concentration of congo red and 0.5 M ethanolic solution of sodium borohydride were prepared. Five test tubes, each containing 5mL of congo red (concentration of 1ppm), 1 mL sodium borohydride, using sterile micropipettes, silver nanoparticles of varying concentration 100, 50, 25 and 10 µL were taken, while one was maintained as blank. The test tubes were incubated at 32ºC for 24 hours. After incubation, samples were withdrawn and analyzed spectrophotometrically using UV-Visible spectrophotometer [8]. The % reduction of congo red dye was calculated by using the following formula: % of Congo red =O.D. of control-O.D. of test sample × 100 / O.D. of control

(iii) Evaluation of effect of synthesized silver nanoparticles on the reduction of malachite green by T. rotundifolia plant extract: In order to assess the catalytic activity of synthesized silver nanoparticless, two reactions were carried out [9]. In the first reaction, 1 mL of malachite green (1×10-4 M) was mixed with 0.2 mL of aqueous plant extract and 1. 8 mL of water and the reaction was monitored after 30 minutes. In the second reaction, 1 mL of malachite green (1×10-4M) was mixed with 0.2 mL of aqueous plant extract and 1.8 mL of synthesized silver nanoparticles (100 mg/mL), and the reaction was monitored after 30 min. The values of absorption maxima of the reaction mixtures were compared with those of malachite green [10].

IV. PHARMACOLOGICAL STUDIES OF SYNTHESIZED NANOPARTICLES

Antioxidant activity:

The synthesized compound (100µL) at different concentrations (31.25µg/mL to 1000µg/mL) was added to 3.9 mL of DPPH in solution (0.025 g/L), and the reactants were shaken vigorously, and then the mixture was incubated at 25°C for 30 minutes. This method helped to determine the antiradical power of an antioxidant by measuring a decrease in the absorbance of DPPH at 517 nm using a spectrophotometer (Genesys 10s UV, Thermo Electron Corporation). Ascorbic acid was used as a reference. Lower absorbance values of reaction mixture indicated higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula. DPPH scavenging effect (% inhibition) = {(A0- A1)/A0) × 100}, where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of the test compound. The experiments were performed thrice and the mean values were taken for the study.

In-vitro Anti-inflammatory activity:

2 mL of Blood sample was collected from the vein of a healthy human volunteer who did not take any NSAIDS for two weeks prior to the experiment, and then the collected sample was stored in a heparinised tube and washed with PBS, and equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) was added and centrifuged at 3000 rpm for 10 minutes (Centrifuge Make: Eppendorf, Model 5810-R). The packed cells were washed with iso saline (0.85% NaCl) suspension. Various concentrations of the synthesized nanoparticles (200, 400, 600, 800, 1000) using plant extracts were prepared in mg/mL using distilled water, and to each concentration, 1 mL of phosphate buffer, 2 mL hypo saline and 0.5 mL of HRB suspension were added. It was incubated at room temperature for 30 minutes and centrifuged at 1500 rpm for 20 minutes, and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm using Micro plate reader (Make: Biotek, Model: Epoch).The experiments were performed thrice, and the mean values were taken for the study. Diclofenac (100µg/mL) was used as reference standard and as a control. The percentage (%) of HRBC membrane stabilization was calculated using the following formula, Percentage of Protection (%) = (100-OD of drug treated sample/OD of Control) × 100.

Antifungal activity:

The synthesized nanoparticles were screened for antifungal activity by agar well diffusion method [11] with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strains such as Candida albicans, Candida tropicalis, Aspergillus fumigatus, Aspergillus niger on PDA plates. An aliquot (0.02mL) of inoculum was introduced to molten PDA and poured into a petridish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. Ketoconazole was used as control after holding the plate at room temperature for 2 hours to allow diffusion of the samples and controls in to the nutrient agar medium. Incubation period of 24-48hours at 28°C was maintained for observation of antifungal activity of the synthesized sample. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm, and the experiment was carried out in triplicates.

V. RESULT AND DISCUSSION

i) Effect of concentration:

Effect of concentration of silver nitrate solution in the formation of silver nanoparticles is shown in Figure 2. As the concentration of silver nitrate solution increases, the intensity of silver nanoparticles band also increases without affecting the band position.

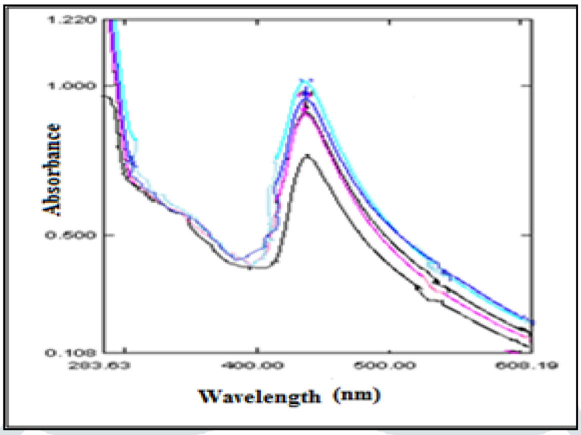

Figure 2 UV-Visible spectrum of synthesized silver nanoparticles at different AgNO3 concentration using T.rotundifolia plant Extract

ii) Effect of pH on bioreduction of Ag+:

According to Evangelin Femila et al., [10] pH plays a vital role in the nanoparticle synthesis, and this factor induces the reactivity of plant extract with silver ions. From Figure 3, it is evident that the formation of silver nanoparticles mainly depends on the pH of the reaction medium. The absorbance value is increased gradually with the increasing of pH range from 5 to 9, suggesting that rate of formation of silver nanoparticles is higher in neutral and basic medium than in acidic medium. In pH 7, a narrow peak nearly at 437 nm for T. rotundifolia is observed. The slow rate of formation and aggregation of silver nanoparticles at acidic medium could be related to electrostatic repulsion of anion present in the solution [12]. At the basic pH range, there is a possibility of Ag+ precipitating as AgOH. On the basis of the results, it could be concluded that the optimum condition for the preparation of silver nanoparticles using the extracts of T. rotundifolia is at pH 7.

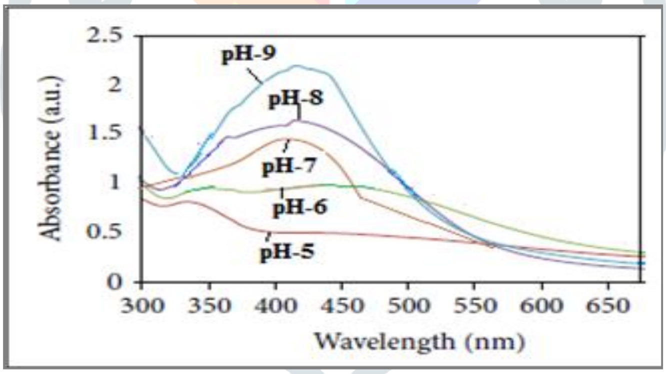

Figure 3 Effect of pHvariation of synthesized silver nanoparticles using T.rotundifolia plant Extract

iii) Effect of time:

The production of synthesized silver nanoparticles was monitored by UV-Vis spectrometer at regular time intervals as shown in Figure 4. The maximum absorbance is noticed at 437 nm for T. rotundifolia. The maximum absorbance is found to be increased almost linearly up to 18 hours of incubation time perhaps due to formation of more number of nanoparticles in the reacting mixture. After 18 hours of incubation, the formation rate saturated indicating the completion of reaction

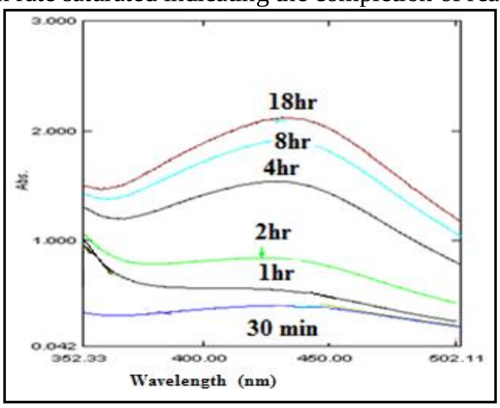

Figure 4 Effect of different time intervals on synthesized silver nanoparticles using T.rotundifolia plant extract

Dye degradation studies of synthesized silver nanoparticles: Evaluation of effect of synthesized silver nanoparticles on the reduction of methylene blue by T. rotundifolia plant extract: Photocatalytic degradation of methylene blue was carried out by using green synthesized silver nanoparticles under sunlight. Dye degradation was initially identified by colour change. The synthesized silver nanoparticles are mixed with methylene blue dye solution and the mixture is exposed to sunlight. After 3 hours of incubation, the colour of the dye solution changed from deep blue to light blue and then changed into light green. The reaction mixture becomes colourless after 73 hours, which indicates the completion of the degradation process. The degradation studies were carried out at different time intervals in the visible regions. The absorption peak at 662 nm for methylene blue dye decreased gradually with the increase of the exposure time. As the absorption peak of methylene blue decreases, and the absorption band of synthesized silver nanoparticles using T. rotundifolia increases at 437 nm. The completion of the photocatalytic degradation of the dye is known from the gradual decrease of the absorbance value of dye approaching the base line and increased peak for silver nanoparticle. While decreasing the concentration of dye, UV spectrum shows a band for silver nanoparticles at 23 hours of exposure time (Figure 5). The percentage of degradation efficiency of silver nanoparticles is calculated as 81% at 73h. The degradation percentage of methylene blue dye increased as increasing the exposure time is shown in Figure 6. Absorption peak for methylene blue dye is at 662 nm in visible region diminishes and finally it disappears while increasing the reaction time. This indicates that indicates that the dye has been degraded.

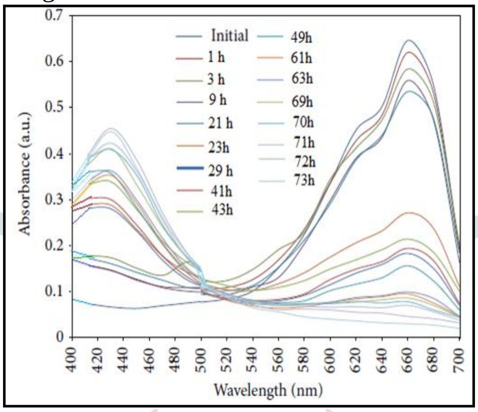

Figure 5 UV-Vis spectrum of photocatalytic degradation of methylene blue dye solution with synthesized silver nanoparticles using T.rotundifolia plant extract at different time intervals

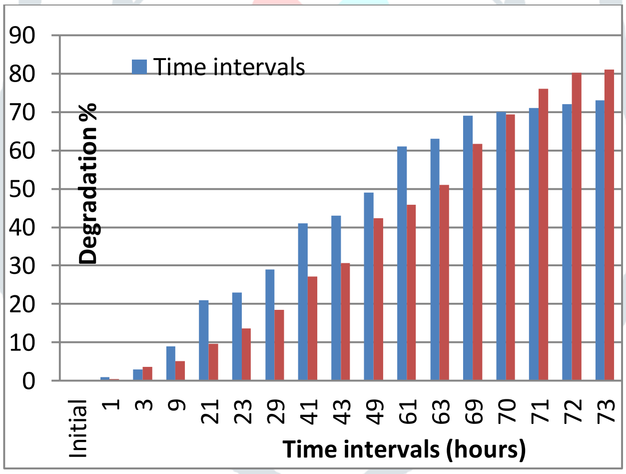

Figure 6 Percentage of methylene blue dye degradation by 10 mg of synthesized silver nanoparticles using of T. rotundifolia plant extract at different time intervals

Evaluation of effect of synthesized silver nanoparticles on the reduction of congo red by T. rotundifolia plant extract: The congo red is a non-biodegradable and toxic azo dye. It originates generally from dyeing industries which pollutes water. This leads to destroy the balance of aquatic environment. In the present investigation, different concentrations of synthesized silver nanoparticles have been applied over the dye for its degradation. After 24 hours of incubation the samples are withdrawn and analyzed spectrophotometrically using UV-Visible spectrophotometer at 400-560nm range. The absorbance readings of the dyes have been noted in Figure 7. A significant decolourization rate is observed and % reduction of the dye is calculated. The significant decolourization rate of congo red dye by synthesized silver nanoparticles using the plant extract of T. rotundifolia was observed within 24 hours of incubation is only 40-50%.

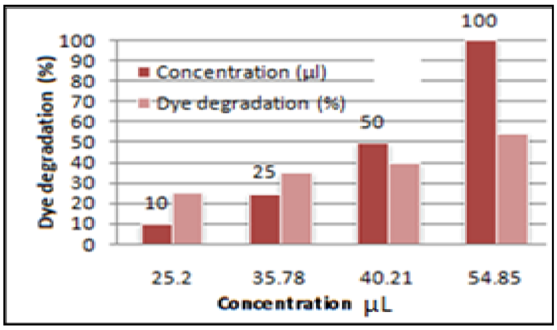

Figure 7 Percentage degradation of congo red dye by using synthesized silver nanoparticles using T.rotundifolia plant extract

Evaluation of effect of synthesized silver nanoparticles on the reduction of malachite green by T. rotundifolia plant extract: The absorption peak for malachite green is at 617 nm. Silver nanoparticles and their composites show great catalytic activity in the area of dye reduction and removal. The reduction of malachite green by the aqueous extract of T. rotundifolia is shown in Figure 8, which represents that the first reaction 2 hours after the addition of the extract to the dye, the absorbance values gradually decreases. The decrease in the absorbance value is indicative of the ability of extract to degrade malachite green. In the second reaction containing dye, silver nanoparticles and the extract, at the end of 2 hours shows a decrease in the absorption of malachite green and increase of SPR peak of silver nanoparticles. This suggests that silver nanoparticles acting as an electron transfer mediator between the extract and malachite green dye and act as redox catalyst, which is termed as electron relay effect [13].

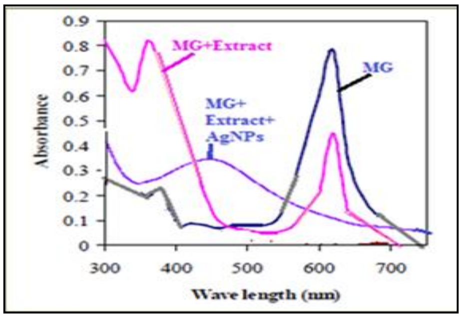

Figure 8 Effect of synthesized silver nanoparticles on the reduction of malachite green using T.rotundifolia plant extract

Atomic fluorescence Microscopy: The surface morphology and roughness of the synthesized silver nanoparticle using plant extract of T. rotundifolia is studied by AFM analysis. Figure 9 shows the AFM image of synthesized silver nanoparticle using plant extract with a scanning area to 0 m to 1.72 µm, and the synthesized silver nanoparticles have rod like structure. The peak height of the synthesized silver nanoparticle is 73.56 nm. The average grain size of the silver nanoparticles synthesized is 20 nm.

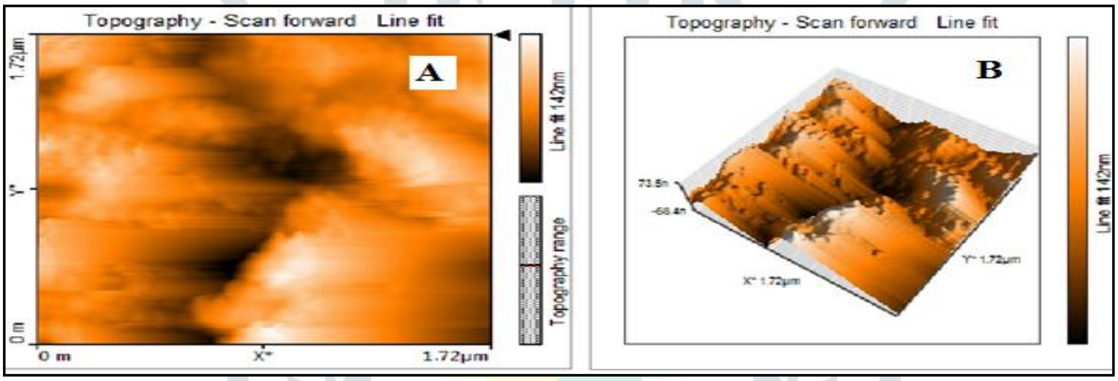

Figure 9 AFM images of synthesized silver nanoparticles using T.rotundifolia plant extract
(A) Lateral micrographs showing uniformly distributed nanoparticles and some agglomeration
(B) 3D image of silver nanoparticle

Cyclic voltammetry: Electrochemical behaviour of silver nanoparticles is determined by CV analysis. Reduction of silver ions into silver nanoparticles in solution is verified by assessing the change in the oxidation states of silver ions. CV is a simple technique to record the redox potential of the species. The selected reduction potential voltage range is from to be -0.6 to 1.2 V. The Glassy Carbon Electrode (GCE) is a working electrode. In cyclic voltammetric analysis, the T. rotundifolia plant extract- free solution makes all the metal ions reduced to lower oxidation state, since there is no possibility for the formation of nanoparticles. On addition of T. rotundifolia plant extract in the reaction medium, the cathodic peak shifts towards the negative potential region, implying that the reduced silver nanoparticles are stabilized by T. rotundifolia plant extract as shown in Figure 10. The extent of decrease in anodic peak current is greater than that of the cathodic peak current due to the fact that the rate of reduction of silver ion may be greater than its oxidation. This might be because of the electron donating methoxy, hydroxyl and amine groups present in T. rotundifolia plant extract which can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AgNPs shows the peaks observed at -0.2 and -0.5V [14].

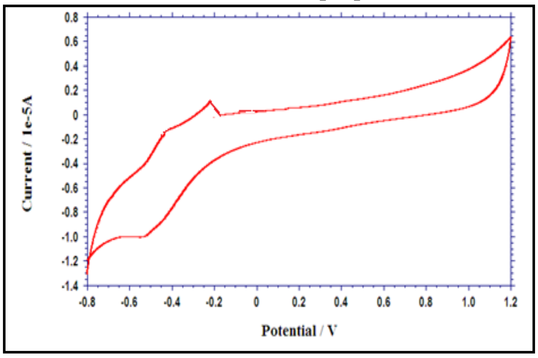

Figure 10 Cyclic voltammograms of silver nanoparticles using T.rotundifolia plant extract

Thermal Analysis (DSC-TGA): The purity and thermal stability of synthesized silver nanoparticles using the plant extract of T. rotundifolia are detected by DSC-TGA analysis. The DSC-TGA of capped silver nanoparticles at optimum reaction conditions is shown in Figure 11. In TGA, the percentage weight loss is from 30ºC to 1000ºC.The initial weight loss about 8.37% at the temperature of 70ºC-180ºC is due to loss the of water molecules from capped silver nanoparticles. The weight loss as shown by the TGA graph proceeds in two steps. The first occurs between 70ºC-180ºC and, the degradation of 42.25% is observed between 180ºC-400ºC. The total weight loss of the silver nano powder is 49.38% and 50.62% of residue remains in the alumina crucible. The degradation which commences at 170ºC is suggested to be the decomposition of bioorganic moiety from capped silver nanoparticles. Thermal degradation of capped silver nanoparticles is not observed from 400ºC to 1000ºC. This shows the stability of silver nanoparticles. The DSC curve shows an endothermic peak at 145ºC and 950 ºC [15].

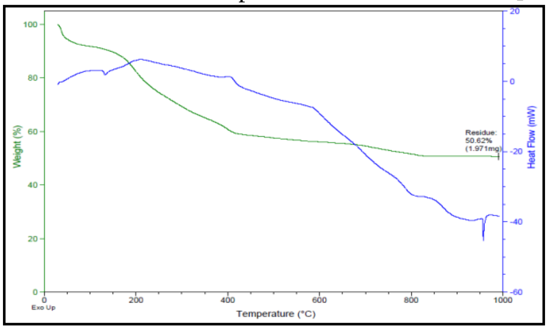

Figure 11 DSC-TGA curve of capped silver nanoparticles prepared using T.rotundifolia plant extract

Particle size distribution analysis: Dynamic light scattering (DLS) is a method that depends on the interaction of light with particles. The light scattered by nanoparticles in suspension will fluctuate with time and can be related to the particle diameter [16]. DLS is a technique used to determine the size, size distribution profile and poly dispersity index of particles in a colloidal suspension. The size of silver nanoparticles analysed shows the "Z" range average values of about 220.9 nm. Poly disparity index (PDI) is a measurement for distribution of silver nanoparticle from 0.0 to 0.299. PDI of less than 0.5 values indicates the aggregation of particles. The size of the synthesized silver nanoparticles using T. rotundifolia is about 83.02 nm which is a slight increase from SEM image of the synthesized silver nanoparticles using T.rotundifolia. Distributions of particle size are shown in Figure 12.

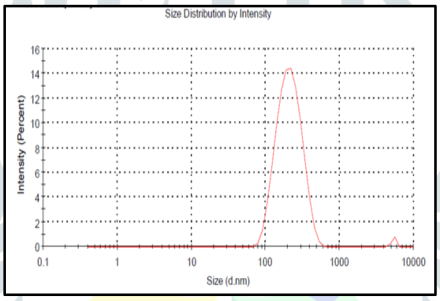

Figure 12 Distributions of particle size for synthesized silver nanoparticles using T. rotundifolia plant extract

Pharmacological Activities of Synthesized nanoparticle Antioxidant activity: The antioxidant activity of synthesized silver nanoparticles is evaluated by using DPPH scavenging assay which is represented in Figure 13. The DPPH values increased in a dose-dependent manner. Phenolic compounds are the major constituents of antioxidants of most plant species and their antioxidant activity is mainly due to their redox properties. As a result, they can act as reducing agents in neutralizing the free radicals [17&18] and the reduction of metal ions to metal nanoparticles. In the present study, green synthesized silver nanoparticles using the plant extract of T. rotundifolia at a concentration of 1000 µg/mL have the radical scavenging activity of 35.96%.

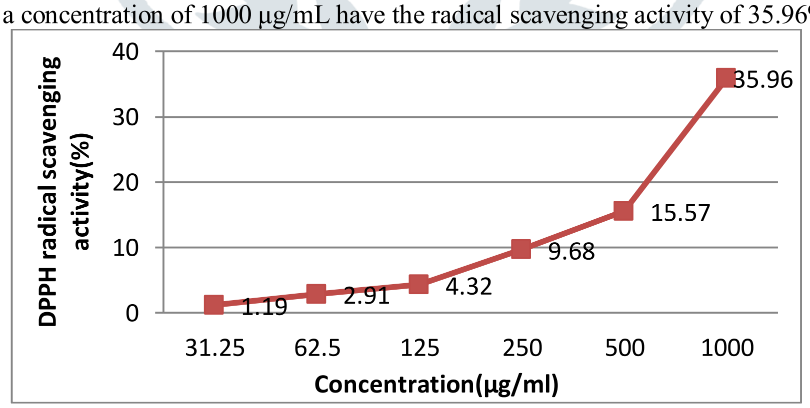

Figure 13 DPPH radical scavenging activity of silver nanoparticles using T.rotundifoliaplant extract

Table 1 DPPH radical scavenging activity of silver nanoparticles using T.rotundifolia plant extract

S. No	Concentration (µg/ml)	DPPH radical scavenging activity (%)Mean±S.E.M
1	31.25	1.19±0.87
2	62.5	2.91±0.35
3	125	4.32±2.74
4	250	9.68±2.27
5	500	15.57±1.05
6	1000	35.96±9.26

Anti-inflammatory activity: Anti-inflammatory study of synthesized nanoparticles is evaluated by Human Red Blood Cell membrane stabilization method as shown in Figure 14. The medicinal use of T. rotundifolia has a good anti-inflammatory activity. As the concentration of the synthesized sample increases, the percentage of inhibition also increases. Synthesized silver nanoparticles show significant anti- inflammatory activity in a concentration-dependent manner. All the results are compared with standard Diclofenac.

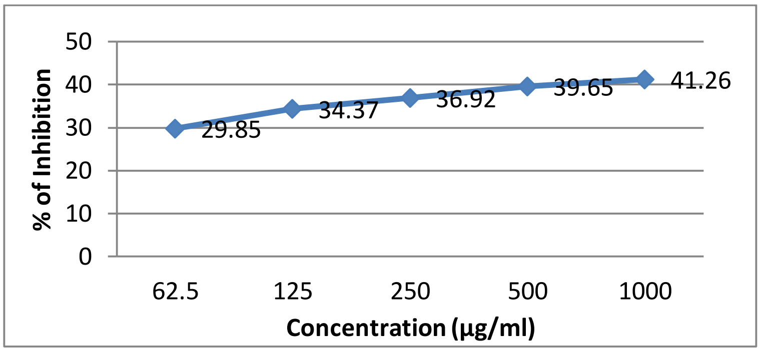

Figure 14 HRBC membrane stabilization activities of synthesized nanoparticles using T.rotundifolia plant extract

Table 2`HRBC membrane stabilization activity of synthesized nanoparticles using T.rotundifolia plant extract

S. No	Concentration (µg/ml)	% of Inhibition Membrane Stabilization Mean± S.E.M
1	31.25	25.88±1.39
2	62.5	29.85±0.50
3	125	34.37±0.46
4	250	36.92±0.29
5	500	39.65±0.07
6	1000	41.26±0.36

Antifungal activity The antifungal activity of prepared silver nanoparticles is found among the four tested different fungal strains *Candida albicans, Candida tropicalis, Aspergillus fumigatus*, and *Aspergillus niger* as shown in Figure 15. Ketoconazole is used as a standard fungicide. Synthesized silver nanopaerticles shows better growth against Candida albicans and Aspergillus fumigates fungal strains. Silver nanoprticles synthesized using T. rotundifolia show resistance against Candida tropicalis, and Aspergillus niger. The Zone of inhibition values are noted in Tables 3.

Table 3 Antifungal activity of synthesized silver nanoparticles against four different fungal strains showing zone of inhibition

Fungal strains	Standard Ketoconazole (10µg/disc)	Zone of inhibition (µg/mL)		
		5	**10**	**15**
Candida albicans	16	8	10	12
Candida tropicalis	14	R	R	5
Aspergillus fumigatus	12	6	8	10
Aspergillus niger	14	R	R	R

VI. CONCLUSION

Synthesized silver nanoparticles using T. rotundifolia when tested for their ability to decolourise the dyes like methylene blue, congo red and malachite green show considerable decolourisation. The photocatalytic studies conclude that synthesized silver nanoparticles have efficiency to degrade the above-mentioned dyes which can find applications in textile industry and water treatment plants. Eco-friendly nanoparticles, bactericidal, antioxidant, anti-inflammatory, wound healing and other medical and electronic applications, make this method potentially exciting for the large-scale synthesis of other nanomaterials.

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