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Synthesis & Bio-Evaluation of 4-Amino-5-Benzyl-2, 4-Dihydro-3H-1, 2, 4-Triazole-3-Thione Capped Silver Nano Particles

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ABSTRACT

Synthesis of 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione Capped Silver NanoParticles by a simple procedure. Spherical shaped Capped Ag-NPs with average size of 14.65 nm are obtained by the treatment of aqueous silver ions with hot ethanolic solution of 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione as Stabilizing/Reducing agent. The Nanoparticles are characterized using TEM, XRD and FTIR studies. The synthesized Nanoparticles were tested for in vitro antimicrobial activity at concentrations of 50, 100, 200 µg /ml. The Nanoparticles showed good activity, nearly equal to the inhibition zone value of ciprofloxacin, against the E.coli bacteria. For the antifungal activity, the compound showed equipotent activity against A.niger.

Keywords – Antimicrobial, Capping Agent, Silver Nanoparticles, Triazole Derivatives.

I. Introduction

Silver is known for its medicinal properties for ages. Ag-NPs are being extensively studied for their medicinal properties. Uniform sized Nanoparticles show definite and remarkable medicinal properties. The size of the Ag-NPs can be controlled by adding capping agents, which prevents agglomeration of formed Nanoparticles. 1,2,4-triazoles and their derivatives are found to be associated with various biological activities such as anticonvulsant [1-2], antifungal [3-5], anticancer [6-9], anti- inflammatory [10-12] and antibacterial properties [13-16]. Several compounds containing 1,2,4-triazole rings are well known as drugs. For example, fluconazole is used as an antimicrobial drug [17], while Vorozole, Letrozole and anastrozole are non-steroidal drugs used for the treatment of cancer[18] and Loreclezole is used as an anticonvulsant. [19]

II. Materials & Methods

2.1 Chemicals Used

Analytical grade $AgNO_3$, AgI and NaI are used. Triply Distilled water is used through out the preparation.

4-amino-5- benzyl-2, 4-dihydro-3H- 1, 2, 4-triazole-3-thione (figure.1) is synthesized in our lab[20] by microwave heating of an equimolar mixture of phenyl acetic acid and thiocarbohydrazide.

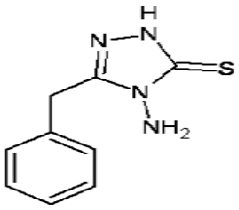


Figure.1

IR-spectra (cm^{-1}) of 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione showed absorption bands at 3309(NH2),3153,3097(NH),1625(C=N),1296(C-N),1045(C=S).

Melting points (m. p) were recorded on Kumar capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded from KBr disks on Thermo Nicolet (Model: 6700) spectrophotometer. The nuclear magnetic resonance (NMR) spectra were recorded on 400 Mhz Fourier transform-nuclear magnetic resonance (Bruker Model: Avance-II) spectrophotometer using tetra methyl silane as an internal standard. The determination of the purity is accomplished by TLC on silica gel plates.

2.2 Synthesis of Capped Ag-NPs

A total of 2.5 mL of 10^{-2} M $AgNO_3$ was added to 75 mL of triply distilled water. After 10 min 2.5 mL of 10^{-2} M NaI was dropped into the solution slowly, yielding a green yellow AgI colloid. A total of 20 mg of $NaBH_4$ was added to the AgI colloidal solution, and the reaction mixture was continually stirred. Immediately after addition of $NaBH_4$, a total

of 5 mL of 10^{-2} M 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (dissolved in hot ethanol) was added as stabilizer to the solution with stirring. The silver colloid was finally obtained. During the whole reaction, the color of the colloidal solution changed from green-yellow to nut-brown, and then to black.

The Nanoparticles are characterized using TEM, XRD and FTIR studies. The synthesized Nanoparticles were tested for *in vitro* antimicrobial activity at concentrations of 50, 100, 200 μg /ml.

2.2 Results and Discussion

Fig-**2.3**, shows the UV-vis spectra recorded from 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-$AgNO_3$ (0.025 M) solution as a function of time of reaction. After addition of $AgNO_3$ solution to the stabilizer, the color changes from dark brown to black. At this stage, formation of metal nanoparticles due to reduction was followed by UV-vis spectroscopy. The generation of color is due to excitation of surface plasmons in metal nanoparticles. The silver surface plasmon resonance was observed at 428 nm which steadily increases in intensity as a function of time of reaction without showing any shift of the Wavelength maximum. This simply indicates longitudinal plasmon vibrations. Also, the plasmon bands are broadened with an absorption tail in the longer wavelengths, which may be due to the size distribution of the particles.

It seems that the present procedure in synthesis of Ag-NP proceeds with a fairly faster pace and reduction of silver ions is complete within minutes. In earlier studies on synthesis of silver nanoparticles employing bacteria, fungi or plant extracts the time required for completion (i.e. complete reduction of Ag-ions) range from 24 to 120 h, and are rather slow. Recently, a good number of works has been done with regard to the reduction of metallic nano particles and the responsible candidate phytochemicals have broadly been ascertained.

2.3 UV-visible, SEM and TEM studies:

Fig- 2.1 shows the UV-vis spectra of silver colloid obtained. The surface Plasmon resonance (SPR) band is broad indicating poly-dispersed nanoparticles. A smooth and narrow absorption band at 428 nm is observed which is characteristic of mono-dispersed spherical nanoparticles. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The surface plasmon resonance (SPR) band (λ max) around 428 nm broadened and slightly moved to the long wavelength region, indicating the presence and formation of silver nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by surface Plasmon resonances (SPR), which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of silver nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species. The surface plasmon absorption of silver nanoparticles have the short wavelength band in the visible region around 428 nm is due to the transverse electronic oscillation.

The TEM images obtained for colloid is shown in figure-**5**. It is clear from the TEM images in figures 4a and 4b that the particle size, nearly spherical particles of average size 14.65 nm is obtained. The typical high resolution TEM image (figure-**2.3**) confirms the particles are spherical in shape.

The Scherrer rings, characteristic of fcc silver is clearly observed, showing that the structure seen in the TEM image are nano crystalline in nature. It is observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under TEM. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times.

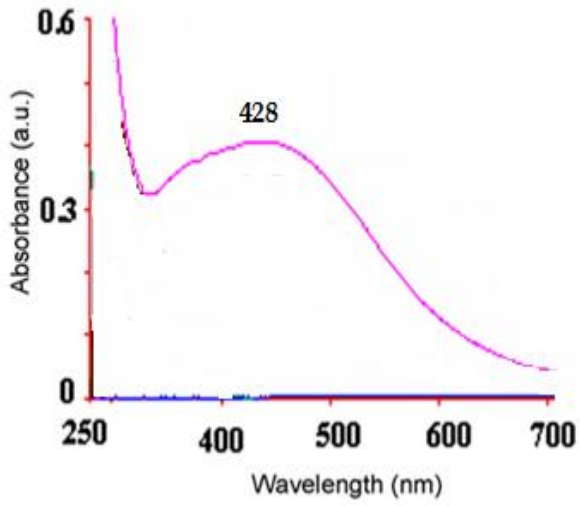


Figure-2.1: UV-visual absorption spectra of silver nanoparticles after reaction

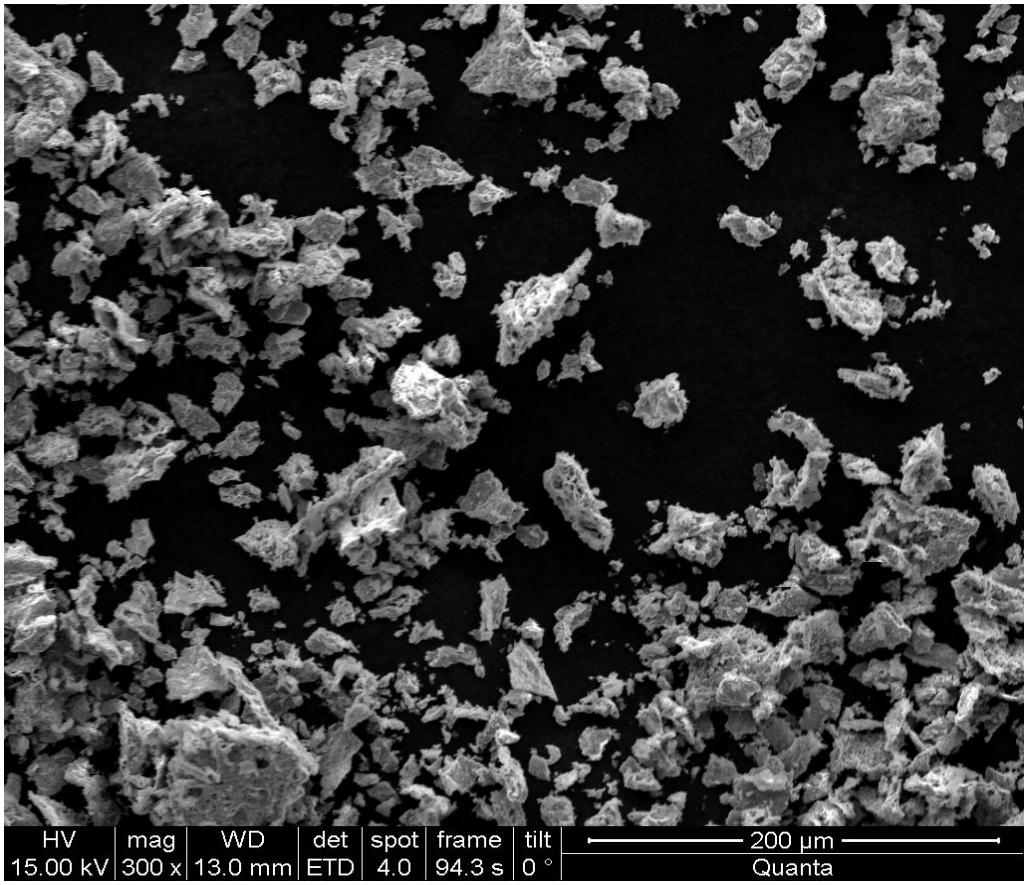
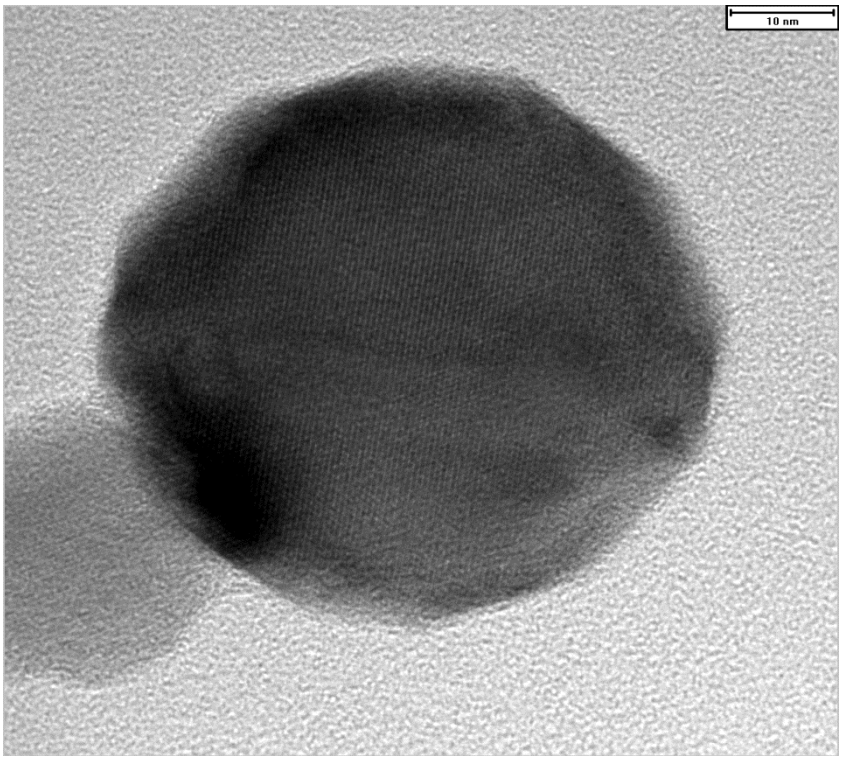


Figure 2.2: Scanning Electron Micrograph of the silver nanoparticles used in this work.



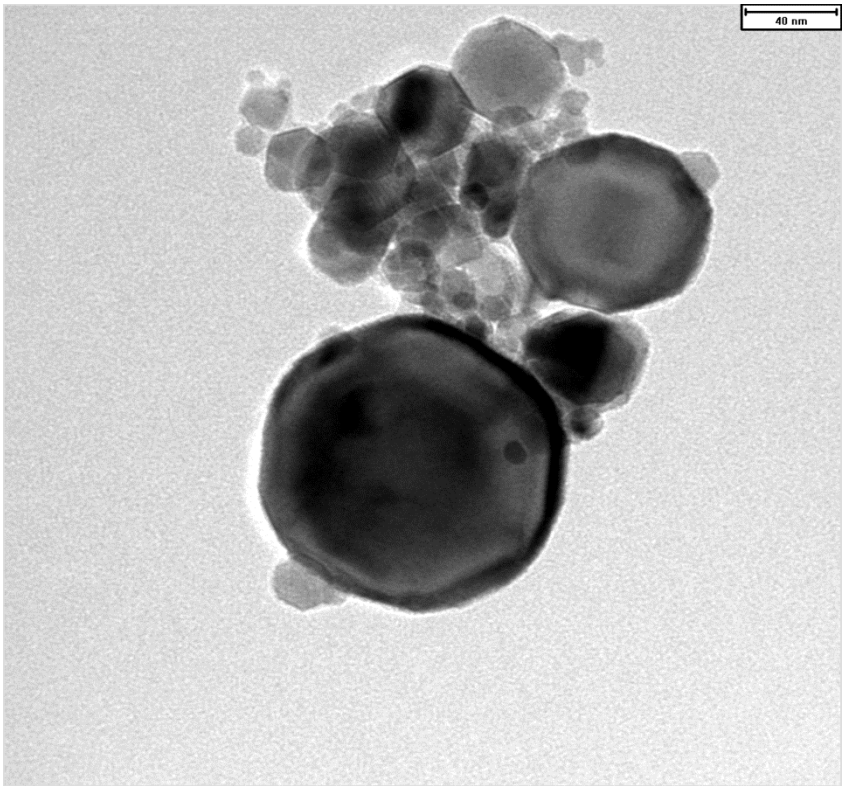


Figure 2.3: Transmission electron micrographs of the silver nanoparticles used in this work. (a) The bar marker represents 40 nm, (b) 10 nm

2.4 XRD and FTIR studies

Fig-**2.4** shows the XRD pattern of Ag nanoparticles obtained. By comparing with standard database values, all the peaks can be indexed to face-centered cubic (fcc) silver crystal structure. Three peaks at 2y values of 38.099, 64.483 and 77.442, correspond to the (1 1 1), (2 0 0) and (2 2 0) planes of silver nanoparticle, respectively. The average crystallite size according to Scherrer equation calculated using the highest peak of the 38.10 is found to be 4.65 nm.

FTIR measurement was carried out to identify the possible functional group responsible for capping and efficient stabilization of Ag nanoparticles synthesized. Figure-**2.5** shows the FTIR spectrum of Ag nanoparticles obtained in this study.

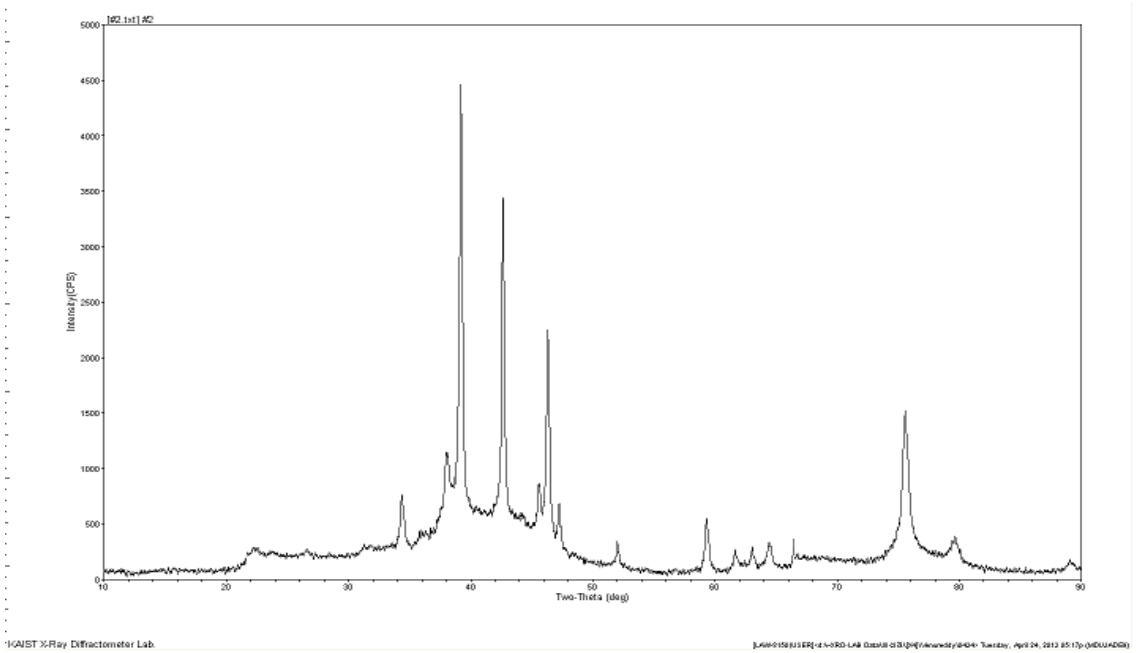


Figure 2.4: X-ray diffraction pattern of Ag-NP at room temperature synthesized by 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione extract with $AgNO_3$ solution.

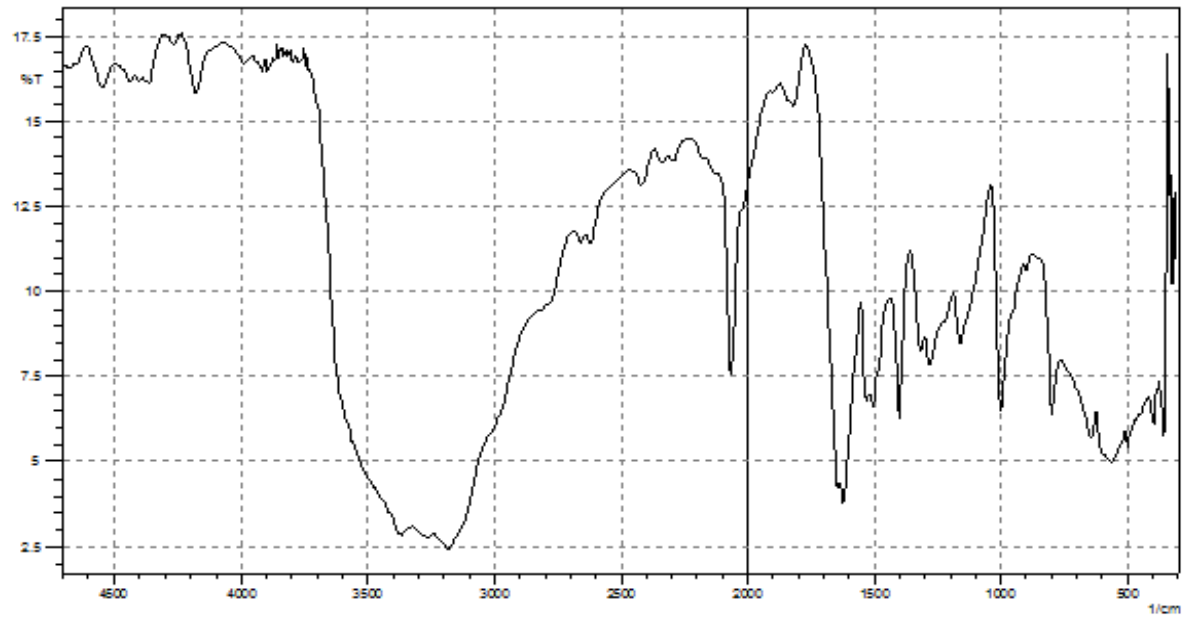


Figure 2.5: FT-IR spectra of 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione mediated silver nanoparticles.

III. Bio-evaluation of 4-amino-5-benzyl-2,4-dihydro-3H-1,2,4-triazole-3-thione Capped Silver Nano Particles:

The synthesized Nano particle was tested for antibacterial activity against bacteria: viz, *E.coli*, *P.aeruginosa*, *S.aureus*, *B.subtilis*, *C.albicans*, *A.niger* at concentrations of 50, 100, 200 µg /ml. The cultures of organisms grown overnight at 37^0C and used for testing the antibacterial activity which was checked employing cup plate method. Nutrient agar medium (Himedia, India) was dissolved in water and pH was adjusted to 7.0. This was then distributed in 20 ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50 µl) was then added aseptically to the agar medium maintained at 45^0C, mixed well and poured immediately in sterilized petriplates. Test solutions

of different concentrations of compounds were prepared in DMSO. After hardening, cups of 8 mm diameter each were cut into agar and 50 µl test solutions of varying concentration (50, 100, 150 and 200 µg/ml) were placed in these cups. The plates were incubated at 37^0C for 24 hours and the diameter of inhibition zone was measured in mm's. Solvent DMSO was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotics Ciprofloxacin & Fluconazole, antibacterial activities inhibition zones of the compounds are presented in Table-1.

3.1 Results:

The synthesized Nanoparticles were tested for *in vitro* antimicrobial activity. The activity was reported by measuring the diameter of inhibition zone in mm, against Gram-positive and Gram-negative bacteria and fungi and is presented in Table 2.

		bacteria				fungi	
Compound	Conc. of Compound	E.coli	P.aeruginosa	S.aureus	B.subtilis	C.albicans	A.niger
Ciprofloxacin	A	0	28	26	25	-	-
Fluconazole	A	-	-	-	-	20	18
Ag-NP Compound	A	7	25	20	22	17	14
	B	7	15	12	15	11	10
	C	1	10	9	9	-	-

A, 200µg/ml; **B,** 100µg/ml; **C,** 50µg/ml

IV. Conclusion

The tested compounds have shown moderate activity against tested bacteria and fungi. However, the compound showed good activity (nearly equal to the inhibition zone value of ciprofloxacin) against to the *E.coli* bacteria. For the antifungal activity, the compound showed equipotent activity against *A.niger*.

V. Acknowledgements

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