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Extracellular Synthesis of Bactericidal Silver Nanoparticles Using Actinobacterium Dagang 5

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Abstract:Biologically synthesised silver nanoparticle using microbes are potential, cost effective and sustainable. Basically actinomycetes are popular for antibiotic and other therapeutic needs. In the present study the silver nanoparticle was synthesised using Actinobacteriumdagang 5 from marine sediments. The silver nitrate was treated with cell free supernatant of Actinobacteriumdagang 5 to synthesis the bactericidal silver nanoparticle and the initial characterisation was performed by observation of color change to intense brown color, UV-Vis spectrum of nanoparticle exhibited an absorption peak at 420nm that indicate surface Plasmon resonance of nanoparticles, X-Ray Diffraction (XRD) patterns showed the distinct peaks, and confirmsthat the nanoparticles are crystalline in nature at average size of 39.28nm. Fourier Transform Infrared Spectroscopy (FT-IR) analysis reveals the evidence, the protein acts as reducing and capping agents. Field Emission –Scanning Electron Microscope (FE-SEM) images showed the formation of nanoparticle. Energy Dispersive X-ray (EDAX) spectra register the presence of silver by showing as major signal. The stability of synthesised nanoparticle was analysed, the zeta potential of our nanoparticle is -21.3mV, which showed nanoparticle are highly stable. The biologically synthesised silver nanoparticle exemplified potential bactericidal efficacy towards the bacterial pathogens. From this study connote that Actinobacteriumdagang 5 is more desirable for the synthesis of Silver nanoparticle by extracellular method in the mode of efficient and eco friendly.
Keywords: Silver nanoparticles, XRD, Antibacterial activity, FE-SEM, Actinobacteriumdagang 5

I. INTRODUCTION

Nanotechnology is upgrading field in science and technology [1]. In which Notably the silver nanoparticle plays vital role in several applications such as bio-labelling, intercalation materials for electrical batteries, bio molecular detection & diagnosis, catalysis, antimicrobials and other therapeutic needs. Marine actinobacteria has high G+C content, parallel they have ability to produce the new chemical derivatives by using its PKS and NRPS. Current urge in the field of medical nanotechnology is to reduce the use of toxic solvents and hazardous reducing agents, finally the regnant belief falls on exploiting the marine actinobacteria in nanotechnology field. In which potential antimicrobial drugs are invaded in the mode of green chemistry approach [2]. It was reported in the earlier research, since from the ancient period the silver used as antimicrobial agent [3]. Whereas silver nanoparticles plays special role in antimicrobial activity with the special characteristics of small size and high surface area to volume ratio [4]. Silver nanoparticle show highest growth inhibitions of pathogens, numerous possibilities are proposed in the mechanism of inhibition. Silver ions have the ability to bind with the negatively charged bacterial cell wall which leads to rupture and denaturation of protein [5]. The silver nanoparticle interacts with DNA and causes the cell death [6]. In other view the blocking of respiration channel or rupture of plasma membrane which leads to depletion of intracellular ATP and cell death [7]. Hence considering the importance of biologically synthesised nanoparticle in pharma industries, in the present study the biosilver nanoparticles are synthesised using *Actinobacterium dagang-5* and characterised. Further antibacterial effiacy of biosilver nanoparticle was evaluated against bacterial pathogens.

II. MATERIALS AND METHODS

A. Biosynthesis of Silver Nanoparticle

The *Actinobacterium dagang-5* strain was isolated from the marine sediment sample from the Arichimunai (9.1794° N, 79.4183° E Gulf of Mannar and their partial 16S rRNA gene sequence was deposited in gene bank the accession number is KM925137. *Actinobacterium dagang-5* strain was inoculated into sterile ISP2 culture medium and incubated at 28°C for 5days at 200rpm. After the incubation period, the cell free supernatant was collected from culture medium by centrifugation at 10 000 rpm for 10mins. The cell free supernatant was used for the synthesis of extracellular silver nanoparticle by mixing 95ml of1 mM $AgNO_3$ and 5ml of CFS and incubated at room temperature in dark condition at 200rpm for 96 h. The color change was noted at specific time intervals (24 hours, 48 hours, 72 hours and 96 hours).

II. CHARACTERIZATION OF SILVER NANOPARTICLE

The synthesized silver nanoparticleswereinitially monitored by visual observation of color change from white to brown. In the bio-reduction process UV-Vis spectrophotometer measurement was performed by UV-Vis double beam spectrophotometer (ELICO SL 210)at200-600nm range.The synthesized silver nanoparticle was freeze dried and taken for further characterization studies. The spectra were evaluated using X-ray diffractormeter (BRUKER, Model-D8 Advance) and Cu-kα radiation 1.5405Å over an angular range of 5 to 80°, and scan at 40kv voltage and a 30mA current. The dried Nanoparticle powder was diluted with Potassium bromide in the ratio of 1:100 and FTIR (Perkin Elmer, Model Spectrum RX I) analysis of biosynthesized silver nanoparticle was performed on the spectrum of GX Spectrometry within range of 400-4000 cm^{-1}. The silver nanoparticle has been further characterized by FE-SEM (EIGMA). For this sample has been prepared by centrifugation of the solution at 10 000 rpm for 10minutes. The pellet was dried in hot air oven at 37℃ for 2days. Later the powder form sample is subjected to FE-SEM analysis by mounting on specimen stubs with double sited adhesive tape coated with platinum in sputter coater and examined the presence of silver metal in the sample was analysed. In addition to this presence of silver particle was analyzed by Energy Dispersive X-ray analysis (EDAX), where the acquisition time ranged from 60 to 100sec and accelerating voltage was 20v. Finally the stability of the nanoparticle was evaluated using Dynamic Light Scattering (DLS) (Malvern Zetasizer) measurement conducted with zetasizer compact scattering spectrometer.

III. ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLE

The antibacterial activity of biologically synthesized silver nanoparticle against bacterial pathogenic organisms such as Klebsiellapneumoniae(MTCC 1687), Proteus vulgaris (MTCC 3160), Salmonella typhi(MTCC 3231), Shigella dysenteriae(MTCC 3642), Vibrio cholerae(MTCC 3906) was investigated by well diffusion method. Pure cultures of the pathogenic bacteria were grown in Muller Hinton broth at 35°C for overnight on rotary shaker at 180rpm. Wells were made onMH agar plate at 6mm in diameter using a gel puncture.Plateswere inoculated with the bacterial pathogens and silver nanoparticles (50µg/ml) at the various concentrations (25µl, 50 µl, 75 µl, and 100 µl) are loaded in each well, the positive and negative controls are also maintained. Plates are incubated at 37°C for 24hrs. After incubation the susceptibility of the test organism was recorded by measuring the diameter of the zone of inhibition.

III. RESULTS AND DISCUSSION

Reporting the result of biosynthesised silver nanoparticle Actinobacteriumdagang 5 by extracellular method, The development of brown color change was observed in the cell free supernatant treated with 1mM $AgNO_3$ at 48 hrs incubation(Fig .1a), Whereas no changes occurred in aqueous $AgNO_3$ without cell free supernatant under the same condition. Thecolorchange indicates the formation of AgNPs by the positive reduction in the culture supernatant. Notably the nanoparticle reaches its maximum intensity after 96 hrsincubation, the reason behind raise of intensity is due to excitation of surface Plasmon resonance factor and reduction capacity of $AgNO_3$ in culture supernatant [8]. The UV- Vis spectra analysis shows strong intense peaks are recorded at 420nm, which indicates the characteristic wavelength of silver nanoparticle (Fig 1b). The reason behind the synthesis of nanoparticle is reduction of metal ions with the help of secondary metabolite present in the cell free supernatant and the hitting of intense peak is due to the excitation of longitudal surface Plamon vibration and nanoparticle superstructure formation [1],[9]-[11].

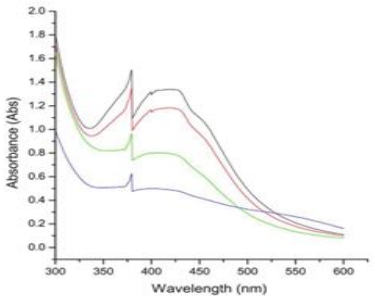

Fig. 1a Occurrence of intense brown color in Cell free supernatant of Actinobacteriumdagang 5. A: 1mM $AgNO_3$, B: Control (No reductant) ; CFS :1mM $AgNO_3$ and 5% cell free extract (Contain reductant), Fig.1b UV-Vis spectra of Silver nanoparticles synthesied using cell free supernatant of Actinobacteriumdagang 5

The biosynthesisednanoparticles are subjected to XRD studies. XRD patterns shows four distinct diffraction peaks in the whole spectrum of 2Θ values ranges from 20 to80. The exact patterns of our samples are of 27.66°, 32.09°, 46.07°, 76°(Fig 2). From these data the synthesised nanoparticle is crystalline in nature and the average size is to be 39.28nm.

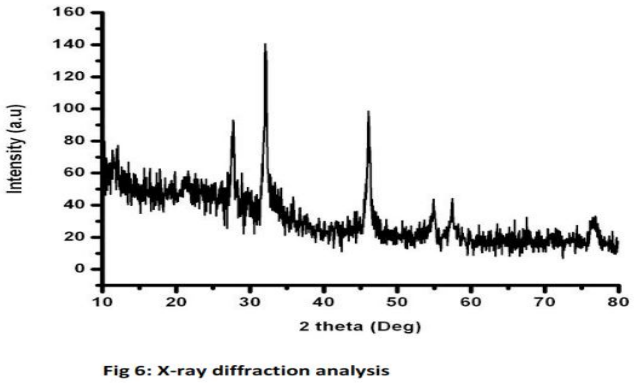

Fig. 2 XRD patterns of the silver nanoparticles synthesized from cell free supernatant of *Actinobacteriumdagang 5*

FT-IR analysis of silver nanoparticles showed intense absorption bands at 3464.47cm^{-1}, 2068.52 cm^{-1},1637 cm^{-1}, 670.45 cm^{-1}(Fig 3). The band 3464.47cm^{-1} and 3434.41 cm^{-1}indicates the presence of amine (N-H) Region, the peak at 2068.52 cm^{-1}showed the carbonyl (C=O) group and the peak at 1637 cm^{-1}and 670.45 cm^{-1}reveals the presence of–C=C- vibration and C-S stretch respectively, which corresponds to heterocyclic compound like proteins.The overall observation confirms the presence of protein in the sample acts as a capping agent for biosynthesised nanoparticles, Based on the earlier reports proteins may binds to the nanoparticles either at the free amine group or at the cysteine residue and caps the nanoparticles which helps to increases the stability of nanoparticle.[9],[12]- [14],

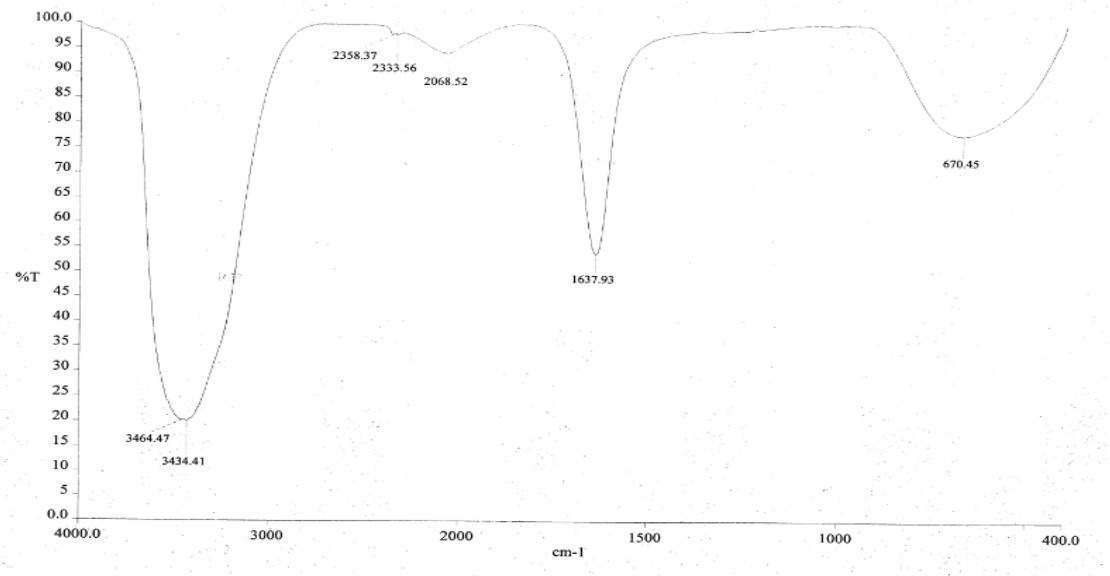

Fig. 3 FT-IR spectrum of the silver nanoparticles synthesized using cell free supernatant of *Actinobacteriumdagang 5*

Fig 4a shows FE-SEM images of the formation of nanoparticle and EDAX spectra are also registered the presence of silver (Fig 4b). The optical peak is observed approximately at 3KeV, which is Surface Plasmon Resonance absorption region of silver nanoparticle. In addition to these peaks, Cl, C, and O atoms are also observed. The presence of these signals due to emission of protein or enzyme present in culture supernatant. The protein has ability to bind with nanoparticle via either free amino groups or cysteine residue [15], [16]. The enzyme present in actinomycetes cell wall also play key role in binding with nanoparticle through electrostatic attraction of negatively charged carboxylate group [2].

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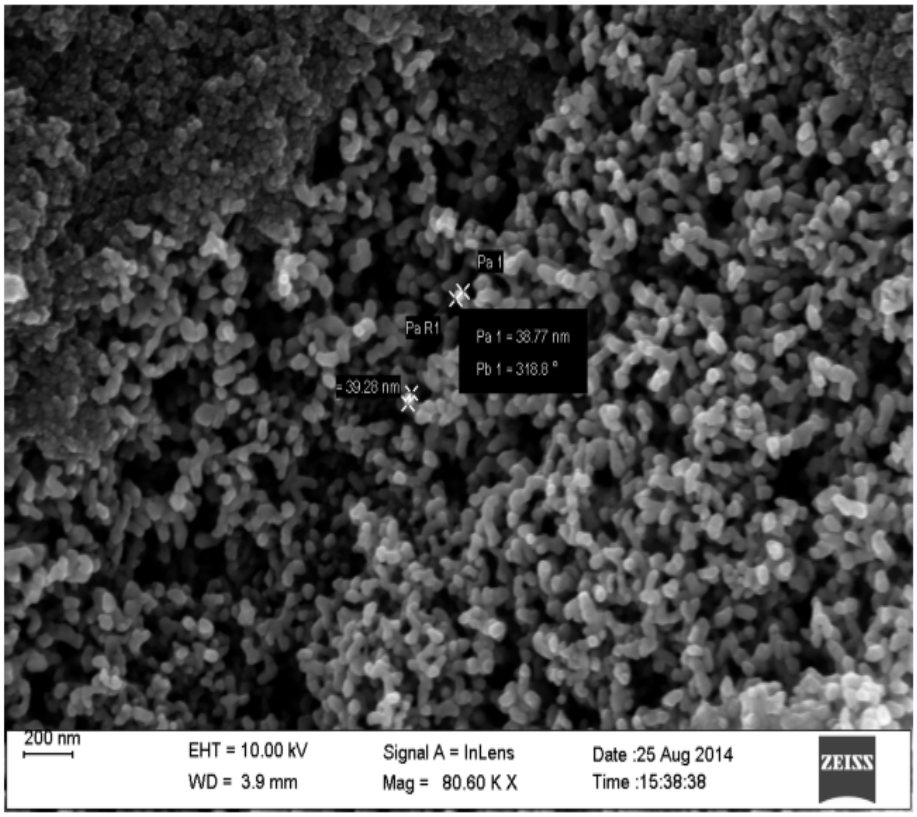

Fig. 4a FE-SEM observations of the silver nanoparticles synthesized by using cell free supernatant of *Actinobacteriumdagang 5* at 200nm scale bar.

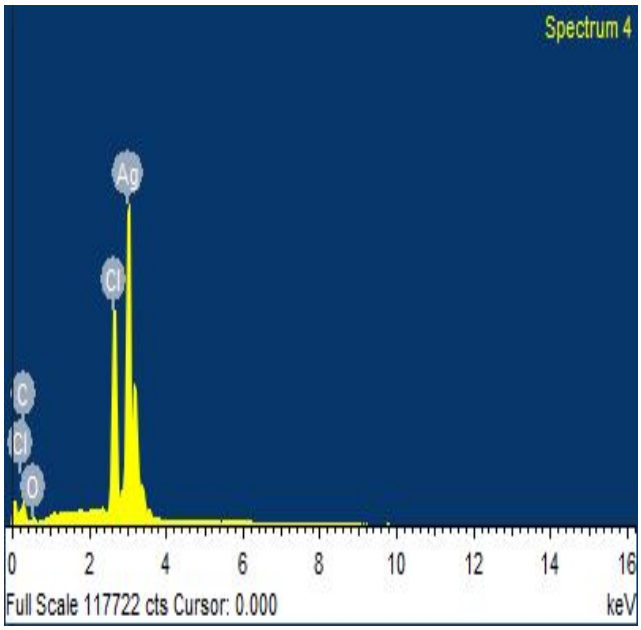

Fig. 4b EDX analysis of the silver nanoparticles synthesized from cell free supernatant of *Actinobacteriumdagang 5.*

Finally the stability of nanoparticle was analysed by the zeta potential the Z average size of our nanoparticle is 445.4nmand the magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticle with Zeta Potential values greater than +25 mV or less than -25 mV have high degrees of stability. The zeta potential of our nanoparticle is -21.3mV, which shows nanoparticle are of highly stable, which are not having tendency to repel each other , it can assemble easily.[1], [3], [17]. The antibacterial activity of

biosynthesized silver nanoparticle was investigated against various bacterial pathogens such as Klebsiellapneumoniae(MTCC 1687), Proteus vulgaris(MTCC 3160), Salmonella typhi(MTCC 3231), Shigella dysenteriae(MTCC 3642), Vibrio cholerae(MTCC 3906)using agar well diffusion method. Table.1 shows bactericidal activity of biologically synthesised silver nanoparticles against bacterial pathogenic organisms. The silver nanoparticle shows highest activity against Proteus vulgaris (20mm) and Vibrio Cholerae (18mm), whereas the lesser activity against Klebsiella pneumonia(12mm) at the low concentration (25µl) of nanoparticle.

Bacterial Pathogens	Antibacterial activity (Zone of Inhibition at mm)			
	25µl	50 µl	75 µl	100 µl
Klebsiella pneumonia (MTCC 1687)	12	18	23	27
Proteus vulgaris(MTCC 3160)	20	26	29	38
Salmonella typhi(MTCC 3231)	14	19	24	29
Shigella dysenteriae(MTCC 3642)	17	21	25	31
Vibrio Cholerae(MTCC 3906)	18	23	27	33

Table I. Antibacterial activity of the biosynthesized silver nanoparticle

The antibacterial activity of silver nanoparticle is due to the penetration of particles into the bacteria. Silver nanoparticle shows highest rate of inhibitions against bacterial pathogens, as various mechanism of inhibition are of described earlier. Silver nanoparticles are of toxic to bacterial cell by disrupting the cell membrane and penetrateinto cytoplasm. Cell death will occur by rupturing cell wall and denaturing the protein or else by depletion of intracellular ATP [6],[7]. Basically the silver ions interact with thiol groups of protein and phosphorus in DNA, which results unwinding of DNA and interfere in DNA replication and cause the cell death.

IV. CONCLUSION

The silver nanoparticles have been synthesised from Actinobacteriumdagang 5 is cost effective and eco-friendly.UV–Vis spectrophotometer, XRD, FTIR, EDAX and DLS techniques have confirmed the reduction of silver ions and the stability of synthesised nanoparticle respectively. The zones of inhibition were formed in the antimicrobial screening test indicates, the efficiency of nanoparticles. The biologically synthesized silver nanoparticles are useful in great extent of medical field.

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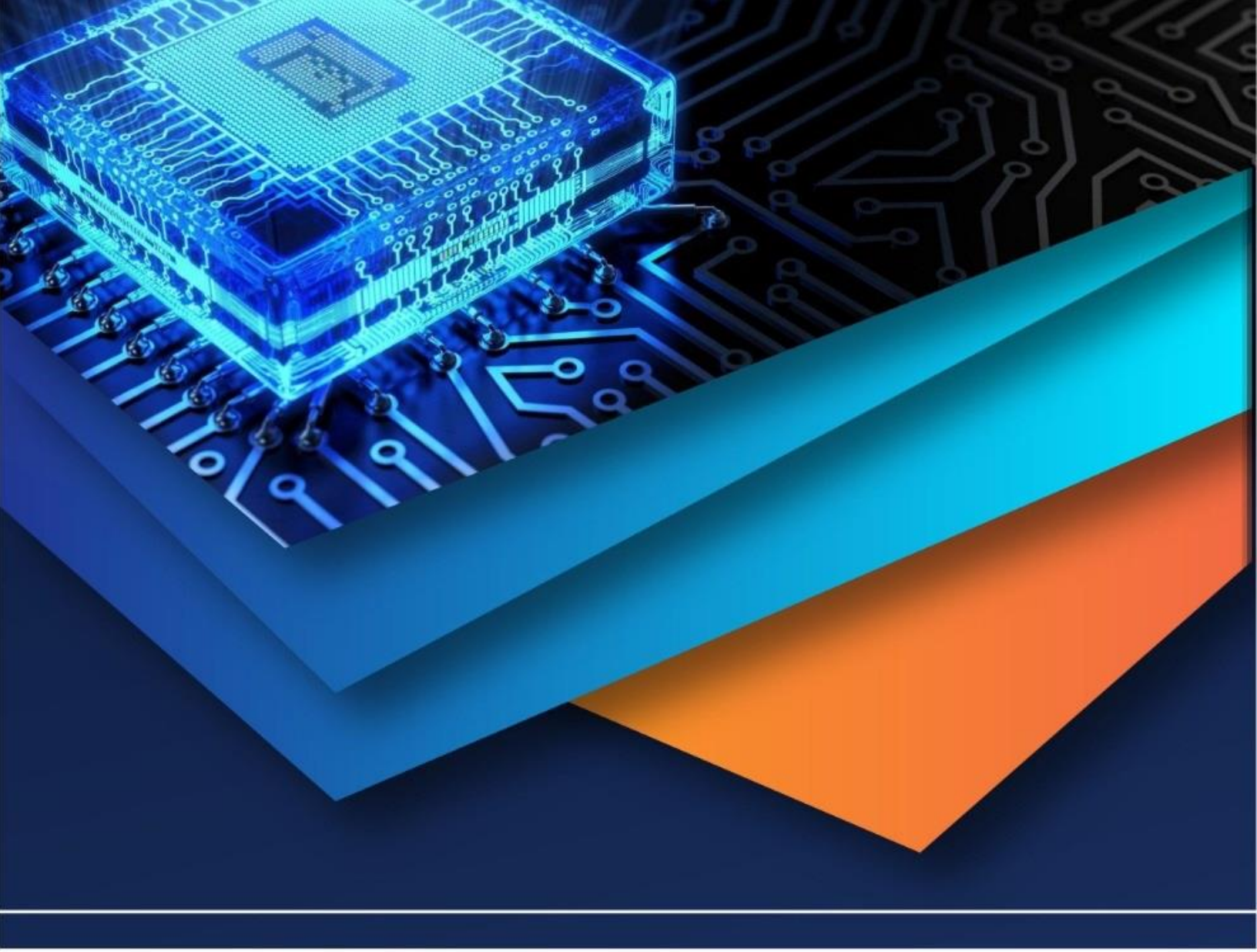

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