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Ecofriendly Synthesis Of Silver Nanoparticles Using Aqueous Leaf Extracts of Hemigraphis Colorata (Blume) Hallier f. And Their Antibacterial Activity

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Abstract: ***The efficient biosynthesis of silver nanoparticles (AgNps) using the leaf extract of Hemigraphis colorata, has been studied and their antibacterial efficacy are evaluated. The plant taken under study is an excellent wound healing plant used to cure anaemia, ulcers and inflammations. The green synthesized silver nanoparticles have been characterized using UV- Visble (UV-Vis) spectrophotometry, X-ray diffraction (XRD), Fourier transform infra-red (FTIR), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). SEM and TEM determine the polygonal nature of the AgNPs with average diameter ranging from 20-50 nm.XRD analysis showed the three strong Bragg reflections at 37.8º, 45.8º and 64.19º that corresponds to the planes (111), (200) and (220) respectively, which is the crystal lattice of silver. Fourier transform infrared spectrometer (FTIR) analysis was carried out to determine the nature of capping agent in the extract. UV-Visible (Vis) spectrophotometer indicates Surface plasmon resonance (SPR) peak was at around 440nm,characteristic of noble metal silver. The biosynthesized AgNPs obtained showed significant antibacterial activities against Bacillus sp., Staphylococcus aureus, Enterococcus sp., Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Moraxella sp. and Serratia plymuthica. The highest antibacterial activity of AgNPs synthesized was found against Pseudomonas aeruginosa (15mm). Thus the study concludes that the biologically synthesized nanoparticles could be of immense use in the medical field for their efficient antimicrobial activity.***

Key words: ***Hemigraphis colorata, silver nanoparticles, SEM, TEM, XRD, FTIR, antimicrobial activity.***

I. INTRODUCTION

Nanotechnology is a fast moving technology applicable in different fields. It has gained importance in medical field for treating dreaded diseases.It is one of the most important fields of modern science dealing with design, synthesis and manipulation of particles (Albecht et al., 2006). It has emerged in recent past for developing facile, green and eco-friendly synthesis of nanoparticles of variable size, shapes, chemical composition and controlled disparity owing to their potential use for human benefits (Swetha et al., 2013). Nano–crystalline silver particles have been found tremendous applications in the fields of high sensitivity bio-molecular detection, diagnostics, antimicrobial, therapeutics, catalysis and micro-electronics (Catauro et al., 2011).Silver is well known for possessing an inhibitory effect towards many bacterial strains and micro-organisms commonly present in medical and industrial processes (Jianget al., 2004). agents. Green synthesis of nanoparticle has been achieved by using environmentally acceptable plant extract and eco-friendly reducing capping agents (Lok et al., 2007).Thus green Nanotechnology encourages not only fundamental but also goal-oriented research in both the academic and industrial fields for the design and development of Green Nanoparticles (Krolikowska et al., 2003).

The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity (Amarendra et al., 2009).Hemigraphis colorata belonging to family Acanthaceae is an herbaceous plant, well adorned for healing the wounds.It possesses various medicinal properties, such as the whole plant or leaves are used to treat fresh wound, cuts, ulcers, inflammation and in folk medicines, it is used internally to cure anaemia. The whole plant of Red-flame ivy is used to stop all external bleeding.Earlier studies on pharmacognstical, phytochemical, antioxidant and anti-elastase activity of the plant were carried out. With this background, our present study is focussed on the biosynthesis and characterization of silver nanoparticles from aqueous leaf extract of Hemigraphis colorata and their antibacterial activity.

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II. MATERIALS AND METHODS

A. *Collection and Preparation of Hemigraphis Colorata leaf Extract*

Fresh leaves of Hemigraphis colorata (Blume) Hallier f. were collected from ATIC medicinal plants, Thrissur, India. Collected leaves of Hemigraphis colorata (Blume) Hallier f. were washed thoroughly and dried using hot air oven at 50ºc. The extraction process was done by adding two gram of powdered dried leaves in to a 250 mL Erlenmeyer flask containing 100ml of deionized water. This mixture was stirredat 80ºc for 45 minutes using magnetic stirrer. The extraction was filtered and supernatant was collected separately and stored at 4°C. Silver nitrate was purchased from Sigma-Aldrich (St Louis, MO, USA) and bacterial strains from microbiology department, Kerala Agricultural University, India.

B. *Synthesis of silver Nanoparticles From Plant Extract*

For the green synthesis of silver nanoparticles, 10mL of the above prepared Hemigraphis colorata leaf extract was dripped slowly into 90 ml of aqueous solution of 1mM silver nitrate. After adding leaf extract into silver nitrate solutions within two mins a visible color change were observed from yellowish brown to reddish brown colour. It showed that aqueous silver ions could be reduced by aqueous extracts of plant parts to generate extremely stable silver nanoparticles. The solution containing silver nanoparticles were separated and concentrated by repeated washing and centrifugation at 16,000 rpm for 20 minutes. The final suspension was dried in hot air oven and nanoparticles obtained were used for further experimental studies.

C. *Characterization of Silver Nanoparticles*

The formation of nanoparticle was confirmed and monitored with the help of various analytical methods. The preliminary characterization of silver nanoparticles was carried out using UV- visible spectroscopy based on optical properties. The reduction of silver ions was monitored by recording the absorption spectra at the range of 400 to 500 nm (Shimadazu, Japan) as a function of reaction time intervals of 1hr, 2hr and 3hr from the initiation of reaction. The AgNps solution was diluted 10 times with deionised water prior to analysis. X-ray diffraction pattern was obtained by using lyophilized powders of silver nanoparticles Phillips PW-1710 automated diffractometer using a Cu Kα radiation in the 2θ range of 10° to 70º operated at a voltage of 40kV and a current of 35mA.This technique was primarily used for the phase identification of a crystalline material and also provides information about the grain size of the silver nanoparticles which can be determined using Debye Sherrer's equation.

$D = K\lambda / \beta \cos\theta$

The Fourier transform infrared spectroscopy (FT-IR) spectroscopic analysis were performed using Shimadazu (Japan) FT-IR spectrophotometer with KBr pellet (1:100 ratio) in the wave number region of 4000 to 500cm^{-1}. Scanning electron microscope (SEM) analysis were carried out using SEM (JEOL-MODEL 639O) at an accelerating voltage of 20KV and Transmission electron microscope (TEM) analysis was done using TEM (JEOL 2100F model) to determine the size and morphology of nanoparticles examined.

D. *Antibacterial Activity By disc-diffusion Method*

The conventional disc diffusion method was used to determine the antibacterial activity of biologically prepared Ag-Nanoparticle. Sterile discs impregnated with20µl(taken from stock solution of 10 mg per ml) biosynthesized AgNPs of plant extract are placed on the surface of the Hi media nutrient agar. During incubation, the antimicrobial agents diffuse from the disc, from an area of higher concentration to an area of lower concentration and inhibit the bacterial growth, and also the measurements can be made of the size of the zone of inhibition around the discs. The antibacterial activities of the AgNPs synthesized were assessed against Bacillus sp., Staphylococcus aureus and Enterococcus sp., Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia plymuthica and Moraxella sp. (Bao et al., 2011).

III. RESULTS

A. *Synthesis of AgNPs*

Green synthesis of silver nanoparticles was carried out by mixing water extract of leaves of Hemigraphis colorata with aqueous solution of 1 mM silver nitrate, a change in colour from yellowish brown to reddish brown was observed. This colour change confirms the reduction of silver ion which indicates the formation of Ag nanoparticles.

B. *Characterization of AgNPs*

The synthesis of the AgNPs in aqueous leaf extract of Hemigraphis colorata was monitored by recording the absorption spectra at a wavelength of 400-500nm, after incubation at time intervals of 1hr, 2hr and 3hr from the initiation of reaction are shown in Graph 1.

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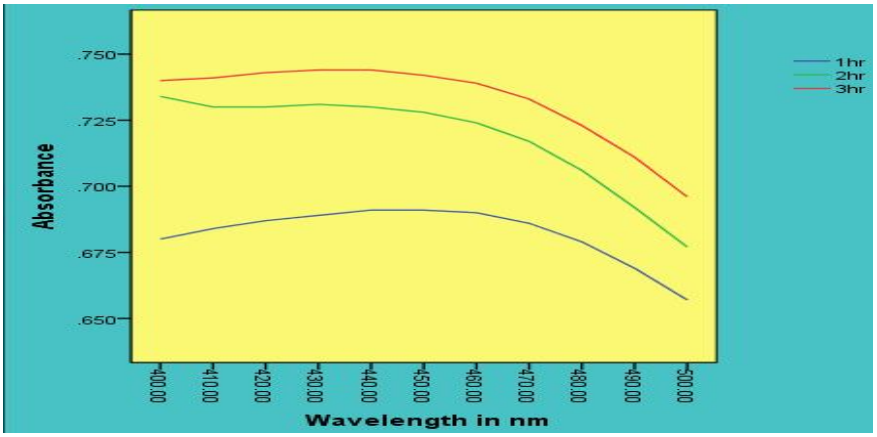
In UV- Vis spectrum, surface plasmon resonance (SPR) peak was observed at around 440 nm, indicating the reduction of Ag^+ ions which further confirmed the formation of silver nanoparticles.

FTIR analysis was carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The observed intense bands were compared with standard values to identify the functional groups. The FTIR spectrum shows prominent absorption bands at 3257, 3070, 2917, 2849, 1738, 1460, 1357, 680 and 526 cm^{-1} as shown in Graph. 2. The band at 1460 cm^{-1} was assigned for N-H stretch present in the amide linkages of the proteins. From FTIR spectroscopic study, it may be concluded that the secondary structure of protein not affected because of their interaction with Ag^+ ions or nanoparticles.

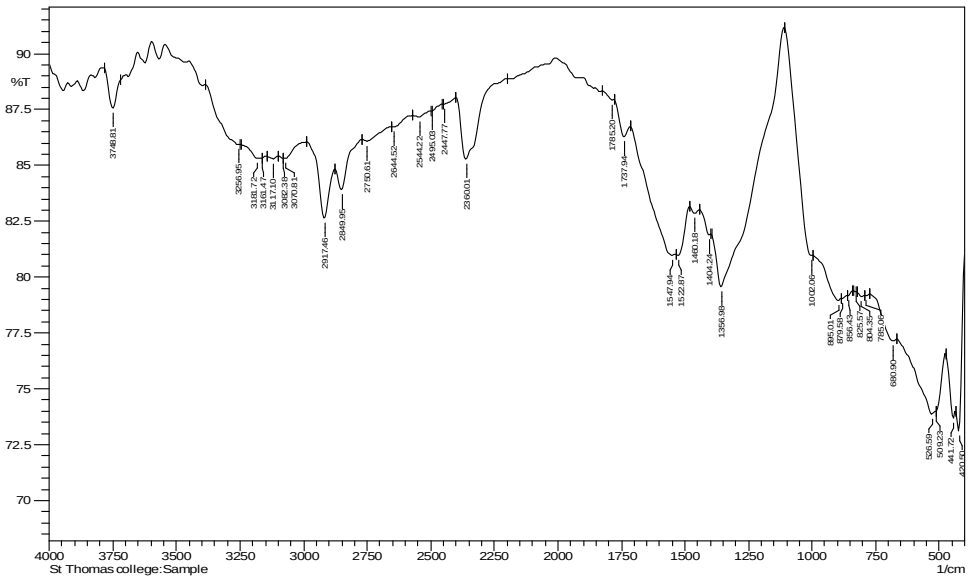
The structure of prepared silver nanoparticles has been investigated by X – ray diffraction (XRD) Analysis and, monitored as shown in the Graph. 3. The XRD study indicates that the formation of silver nanoparticles. The diffracted intensities were recorded from 3º to 70º. Three strong Bragg reflections at 37.8º, 45.8º and 64.19º corresponds to the planes (111), (200) and (220) respectively and compared with standard silver values (Wang *et al*., 2005). Considering the peak at 45.8°, average particle size is calculated as ~27.64. using Debye-Scherrerformula.

SEM technique was employed to visualize the size and shape of silver nanoparticles as in fig no.1. The shape of the silver nanoparticles was found to be polygonal. TEM provides further insight into the morphology and size details of the synthesised AgNPs. The TEM images at different magnifications and are depicted at fig.2. The TEM micrograph with average diameter ranging from 20 nm –50 nm as shown in the graph.4.

Graph 1. UV-Vis spectra analysis of AgNPs of leaf extract *Hemigraphis colorata*



Graph 2. Determination of Silver nanoparticles using FTIR technique



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Graph .3 Determination of AgNps of leaf extract of Hemigraphis colorata using XRD

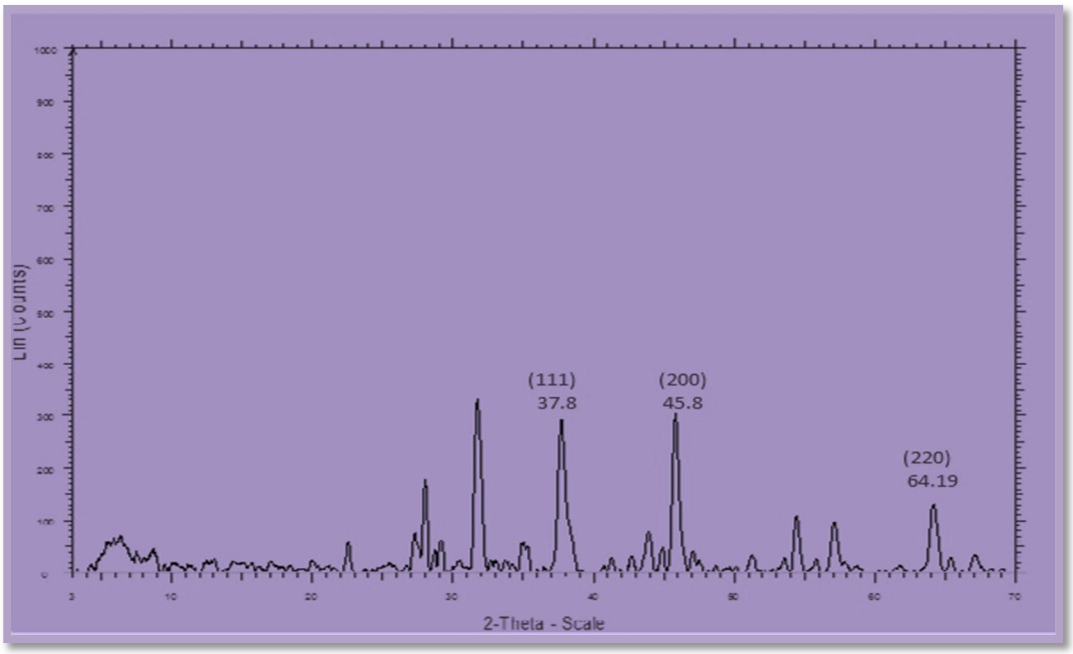


Fig no.1. SEM Micrograph of AgNps of the leaf extract of Hemigraphiscolorata

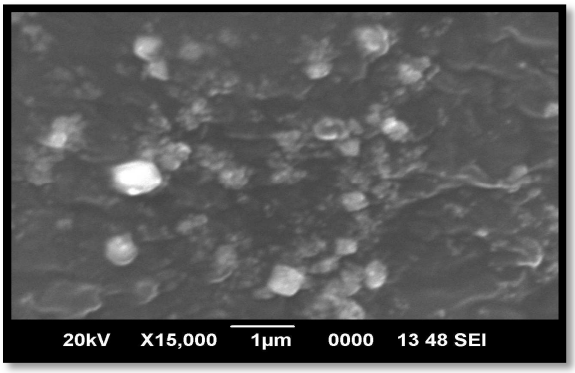
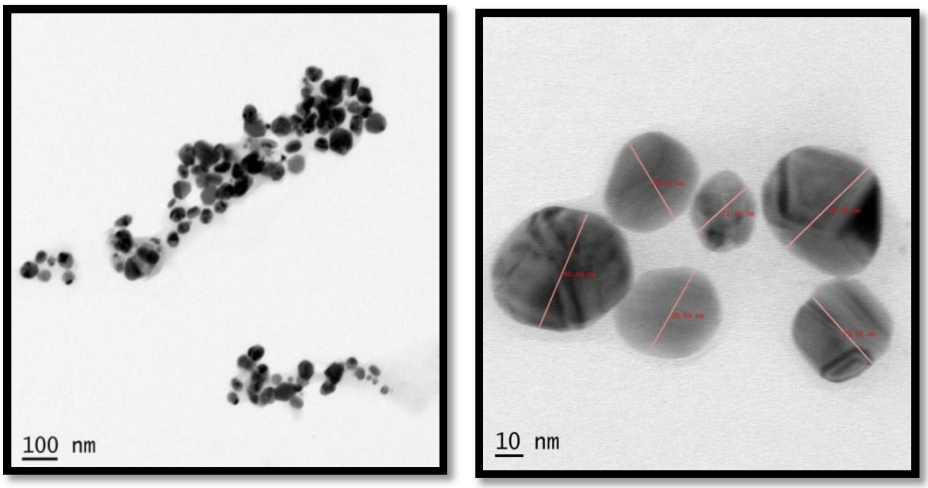


Fig no. 2.TEM Micrograph of AgNps of the leaf extract of Hemigraphiscolorata



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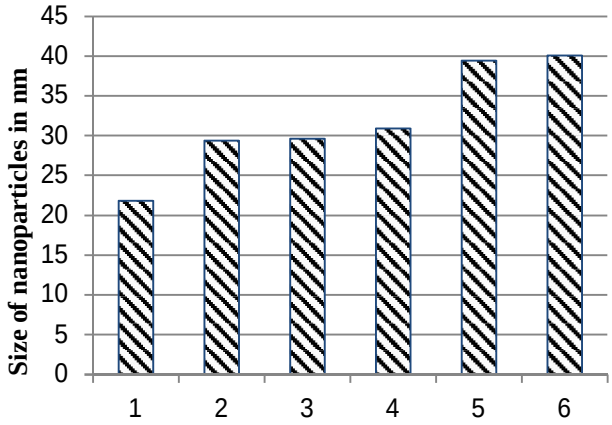
Graph no.4.Showing Size of silver nanoparticles using leaf extract of *Hemigraphis colorata*



C. Antibacterial Activity

Antibacterial activity of biosynthesized silver nanoparticles of aqueous from Hemigraphis colorata was assayed against eight bacterial strains, three gram positive (Bacillus sp., Staphylococcus aureus and Enterococcus sp.) and five gram negative bacteria (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia plymuthica and Moraxella sp.) Among bacterial strains, Pseudomonas aeruginosa are highly sensitive to silver nanoparticles of Hemigraphis colorata and Escherichia coli showed least sensitive when compared with all other bacterial strains.The antibiotic disc Gentamicin taken as positive control showed a significant inhibitory zone as shown in table no.1. The deionised water taken as negative control, which did not showed any zone of inhibition. The P value less than 0.01 is found to be significant using the graph pad MSTATC software.

Table no. 1 Zone of inhibition of silver nanoparticles of Hemigraphis colorata against various pathogenic bacteria

Bacterial strains	AgNps (A)		Gentamicin (B)	
	Average Zone in mm	Pvalue	Average Zone in mm	Pvalue
Bacillus sps.	14	<0.01	30	<0.01
Staphylococcus aureus	11	<0.01	18	<0.01
Enterococcus	12	<0.01	22	<0.01
Klebsiella pneumoniae	9	<0.01	20	<0.01
Escherichia coli	8	<0.01	20	<0.01
Pseudomonas aeruginosa	15	<0.01	25	<0.01
Serratia plymuthica	10	<0.01	20	<0.01
Moraxella	11	<0.01	28	<0.01

IV. DISCUSSION

The biosynthesized nanoparticles from the aqueous leaf extract of Hemigraphis colorata (Blume) Hallier f were characterized to determine the shape, size and nature of crystalline silver nanoparticle using various techniques. The nanoparticles were primarily characterized by UV- Vis spectroscopy, which was proved to be a very useful technique for the analysis of nanoparticles. This

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technique revealed that the maximum absorbance of AgNps was observed at 440nm, which is the characteristic of noble metal silver. The FTIR spectrum shows prominent absorption bands at 3257, 3070, 2917, 2849, 1738, 1460, 1357, 680 and 526 cm^{-1}.This analysis reveals surface capping of protein and aromatic compounds with the AgNPs, for the stabilization of nanoparticle. So the size of biosynthesized AgNPs were found to be larger (100-200nm) as determined in SEM analysis. Thus TEM and XRD analysis were done in order to determine the actual size of AgNPs which was found to be between 20nm-50nm. The XRD peaks at 2ɵ range of 30-70º correspond to (111), (200) and (220) reflection planes that indicate the structure of metallic silver. Considering the peak at 45.8˚ showing maximum absorption, average particle size is calculated using Debye-Scherrer formula was ~27.64. SAED analysis helped to visualize the crystal planes of biosynthesized silver nanoparticle in concentric rings. The effective bactericidal studies reveal that silver nanoparticles synthesized have effective The highest antibacterial activity of silver nanoparticles was found against Pseudomonas aeruginosa (15mm) and lesser activity against Escherichia coli (8mm).

V. CONCLUSION

The rapid biosynthesis of silver nanoparticles using the leaf extract of Hemigraphis colorata (Blume) Hallier f. is an economical, eco-friendly and efficient process. UV-Visible spectrophotometer, FTIR, XRD, SEM and TEM techniques have confirmed the bio-reduction of silver nitrate to silver nanoparticles. The SEM micrographs have showed the synthesised nanoparticles were of polygonal shape. The FTIR study suggests that the protein might play an important role in the stabilization of silver nanoparticles. UV-Vis spectroscopy reveals surface plasmon property of nanoparticle, while XRD peaks and TEM images reveals the nano nature of the prepared samples and the size estimated to be between 20nm- 50nm.Further studies can be focussed on the extraction and identification of the compounds present in the extract which responsible for the reduction and stabilisation of nanoparticles.

VI. ACKNOWLEGMENT

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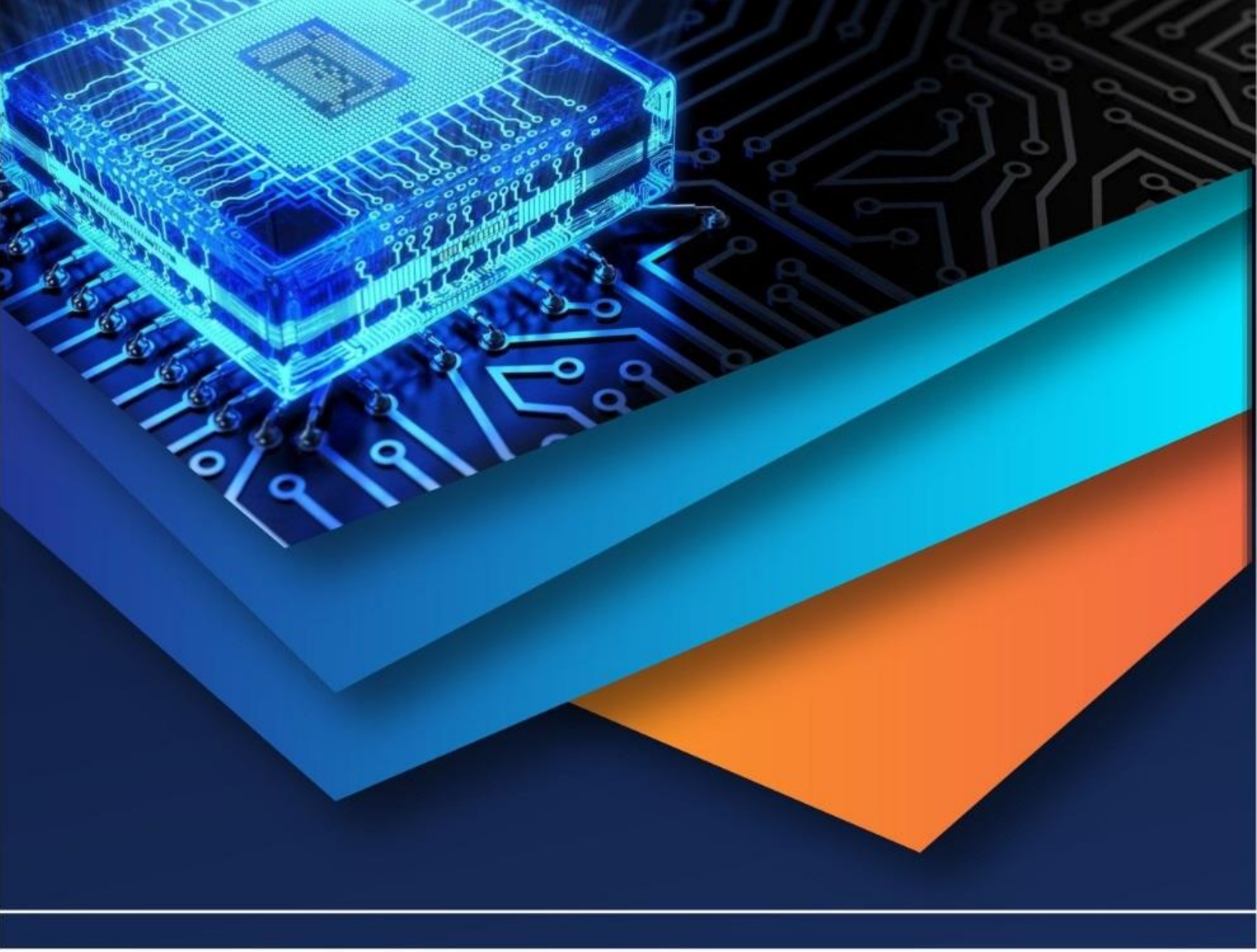

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