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Green Synthesis and Characterization of Zinc Oxide Nanoparticles using Leaves Extract of Aristolochia Bracteolate and its Antimicrobial Activities

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Abstract: ***The paper reviews synthesis of ZnO nanoparticles using Aristolochia bracteolata leaves extract. The Zinc oxide nanoparticles were characterized using the following methods. Biosynthesized ZnO nanoparticles were primarily confirmed by change in colour from brown to white. Ultraviolet-Visible Spectroscopy of the ZnO nanoparticles showed surface plasmon resonance (SPR) peak at 377 nm. Fourier Transform Infrared Spectroscopy was used to key out the specific functional groups present in the leaves extract. The X-ray powder diffraction the structural phase of the ZnO nanoparticles was found in the form of face centered cubic (FCC). The particle size of the synthesized Zinc oxide nanoparticles has confirmed. Field Emission Scanning Electron Microscopy analyses revealed that the synthesized ZnO nanoparticles were shape and size was confirmed and the Energy Dispersive X-ray analysis spectrum showed peaks for the presence in the range 8.5 keV. The synthesized nanoparticles were analysed to know the average size 50 nm were confirmed. The both gram positive and gram negative bacteria such as Staphylococcus epidermis, Bacills subtilis and Klebsiella pneumonia were experienced to analyse the antibacterial activity of the ZnO nanoparticles. To analyse the antifungal activity of the ZnO nanoparticles with the help of fungi called Candida albicans and Candida vulgaris.***

Keywords: Aristolochia bracteolate, Antibacterial activity, Antifungal activity, FTIR, UV, ZnO nanoparticles.

I. INTRODUCTION

Nanotechnology has tremendously enhanced and bred to expand several technologies [1]. The nanoparticles have dimension amid 1 to 100 nm and due to small size and high surface area they grab importance that resulted to unique properties [2]. As Zinc oxide is safe and biocompatible that would be apt in medical applications obviously without overlays. In future, the Zinc oxide can create a plenty of research fields because of its special properties [3-5]. At the moment in time, nanoscience and nanotechnology are extensively applied in several fields especially in biomedical, cosmetic, pharmaceutical, sensor, electronic, antibacterial, water purification, environmental, catalytic and material application. The size morphology and crystallinity of the nanomaterial can significantly impact their catalytic, magnetic, electronic and optical properties [6]. The synthesization of metal and metal oxide nanoparticles are using physical, biological, chemical and very recent green approaches [7]. Currently in nanoscience and nanobiotechnology fields, green synthesis of metal nanoparticles is an interesting aspect and there is an evolving attention to biosynthesis the metal nanoparticles using living organism. Plants seem to be the best candidate among these organisms and are well suitable for large scale biosynthesis of nanoparticles. Nanoparticles which are produced by plants are more stable and is faster synthesis rate as compared to other organisms. Additionally, the nanoparticles are more dissimilar in shape and size in comparison with those produced by other organisms [8]. Due to its anthelminthic activity and trypanocidal effect. Aristolochia bracteolata also known as “worm killer”. The plant Aristolochia bracteolata belongs to the family called Aristolochiaceae. It is a perennial herb growing between 10-60 cm tall. The plant has been used as an infusion of dried leaves a best remedy for intestinal worms, insect bites and skin itch [9,10]. Hence, the present study reported the synthesis of Zinc oxide nanoparticles using aqueous leaf extract of Aristolochia bracteolata and its activity against various bacteria. The structure, size and morphology of synthesized product were explored by the standard characterization techniques. Here we report a green and eco-friendly route for the synthesis of ZnO nanoparticles using aqueous extract of Aristolochia bracteolate leaves extract, UV-Vis spectroscopy (UV), Fourier Transform

Infrared spectroscopy (FTIR), X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM) Energy Dispersive spectroscopy (EDAX) and Dynamic Light Scattering (DLS). The antibacterial and antifungal activity of Zinc nanoparticles was determined using the disc diffusion method.

II. EXPERIMENTAL

A. Collection of Sample

Fresh leaves of Aristolochia bracteolata were collected from Trichy as shown in Fig.1. The collected leaves were washed completely using double distilled water and were made to dry in air at room temperature.

Figure.1 Aristolochia bracteolate

B. Chemicals

The procurement of Zinc oxide were from Sigma Aldrich.

C. Preparation Of Aristolochia Bracteolata Leaves Extract

The thoroughly washed leaves of 10 gram were dried and ground into fine powder and at 60°C the powder were immersed in 100 ml of double distilled water for 15 minutes. With the help of Whatman filter paper, the extract was filtered and stored in a cool and dry place.

D. Synthesis of Zinc Oxide Nanoparticles

The addition of plant extract with 1mM Zinc oxide solution in the ratio of 1:9 aqueous leaf extract and Zinc oxide solution were used. The change in colour of the solution specified in the presence of Zinc oxide nanoparticles as shown in Fig.2. At rpm of 8000 for 15 minutes of centrifugation, the samples were purified.

Figure. 2 Leaves extract and the colour change of after the addition of ZnO

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III. CHARACTERIZATION OF ZNO NANOPARTICLES

A. UV-Vis Spectroscopy Analysis

UV-Vis spectroscopy (UV-VISIBLE SPECTROPHOTOMETER LAMBDA 35 PERKIN ELMER) has confirmed the existence of Zinc oxide nanoparticles by which range between 190 nm to 1100 nm. By surface plasmon Resonance effect, the determination of UV-Vis spectral gives us the insight on the actual formation of the Zinc oxide nanoparticles.

B. Fourier Transform Infrared Spectroscopy Analysis

With the help of FTIR SPECTRUM 1000 PERKIN ELMER SPECTROMETER the FTIR analysis has been performed at range around 400-4000 cm^{-1}. The presence of functional groups were found in the sample by using FTIR spectroscopy.

C. X- Ray Diffraction Analysis

XRDX "X" PERT PRO Diffractrometer is utilized to analyse powder XRD patterns on ZnO nanoparticles.

D. Field Emission Scanning Electron Microscopy and Energy Dispersive Spectroscopy

FESEM images were recorded by using FEI QUANTA-250 FEG instrument. An energy dispersive spectroscopy BRUKER analysis was performed on the prepared sample for qualitative elemental analysis.

E. Dynamic Light Scattering

Nanoplus has been used to evaluate the average particle size of the synthesized ZnO nanoparticles.

1) Antibacterial activity

a) Collection of test Organisms: To examine the antibacterial activity of Zinc nanoparticles, two gram positive bacterial strains Staphylococcus epidermidis (MTCC 737) and Bacillus subtilis (MTCC 2451). one gram negative bacterial strains Klebsiella pneumonia (MTCC 3384) were prepared as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

2) Screening of Antibacterial Activities

a) Antibacterial activity of Zinc Nanoparticles (Disc Diffusion Method): Antibacterial activity of Zinc nanoparticles was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Amoxicillin as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

3) Antifungal Activity

Screening of Antifungal Activities

a) Culture Media: The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

b) Inoculum: The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^{5} CFU/ml.

c) Fungal Strains Used: The clinical fungal test organisms used for study are *Candida albicans* (*MTCC*-3498) and *Candida vulgaris* (MTCC 227), were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

d) Determination Of Antifungal Activity: Antifungal activity of sample was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Fluconazole as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.

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IV. RESULTS AND DISCUSSION

A. UV-Visible Spectroscopy

The UV-Visible Spectrophotometer has confirmed the green synthesis of ZnO nanoparticles. In UV-Vis Spectrophotometer shows the ranges around 200-1200 nm as shows in the Fig.3. The reaction mixture consists of aqueous leaf extract of Aristolochia bracteolata and Zinc oxide solution. A colour change was happened from yellowish brown to white after an hour and the observed colour change clears the formation of ZnO nanoparticles. The UV-Vis Spectra biosynthesized Zinc oxide nanoparticles using Aristolochia bracteolata was observed the maxima of absorption peak at 377 nm and the sharp absorbance peak was monitored in the UV-Vis Spectrum [11].

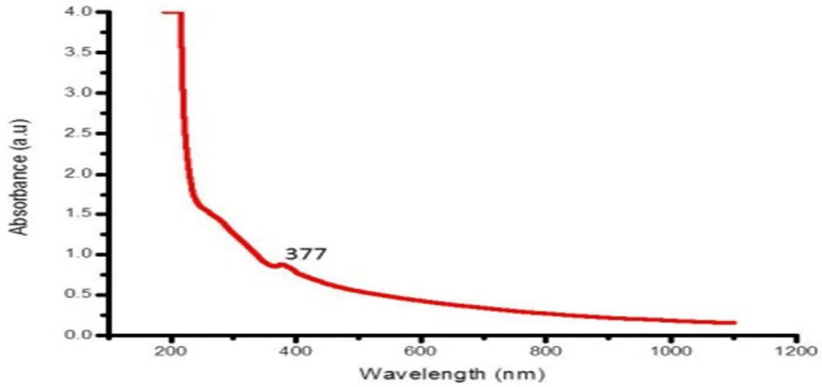

Figure.3 UV-Visible Spectroscopy

B. Fourier Transform Infrared Spectroscopy

The FTIR Spectrum of Zinc oxide nanoparticles absorbs at the O-H alcohol appears in the spectrum as medium band extending from 3369 cm^{-1}. The very broad O-H stretch band is absorbed that the compound is an Carboxylic acid 2923 cm^{-1}. This weak to medium C=C stretch band are 1600 cm^{-1}. An absorption peak found at 1384 cm^{-1} corresponds to C-N stretching. The prominent levels of doublet absorption observed at 1037 cm^{-1} and 1031 cm^{-1} reveal the presence of C-N Stretching Si-O stretching. The band observed at 871 cm^{-1}, C-O bending Vibrations at 700 cm^{-1} Si-O bending at 535 cm^{-1} as shown in Fig.4. FTIR analysis confirmed that the functional groups [12].

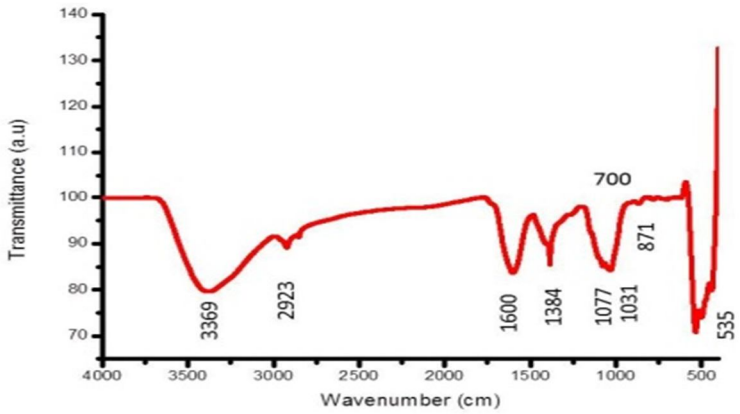

Figure.4 Fourier Transform Infrared Spectroscopy

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C. X-ray Diffraction analysis

The measurement of XRD pattern of samples was used to identify the crystalline structure of ZnO nanoparticles in 2θ range 10-80°. The crystalline structure of ZnO nanoparticles was keep maintained at room temperature and at 90° *C* based on the results. The peaks at 31.78, 38.44, 36.28, 47.55, 56.62, 62.83 and 67.96° equals (100), (002), (101), (102), (110), (103) and (112) crystal planes respectively, which correlate with wurtzite crystalline with hexagonal structure (JCPDS NO: 36-1451). The XRD pattern contains no extra peak confirmed that ZnO nanoparticles synthesized are pure and there is no impurities is it. Also, it can be concluded that Zinc oxide has a good degree of crystalline structure due to the presence of sharp and narrow peaks in the XRD spectrum as shown Fig.5 [13,14].

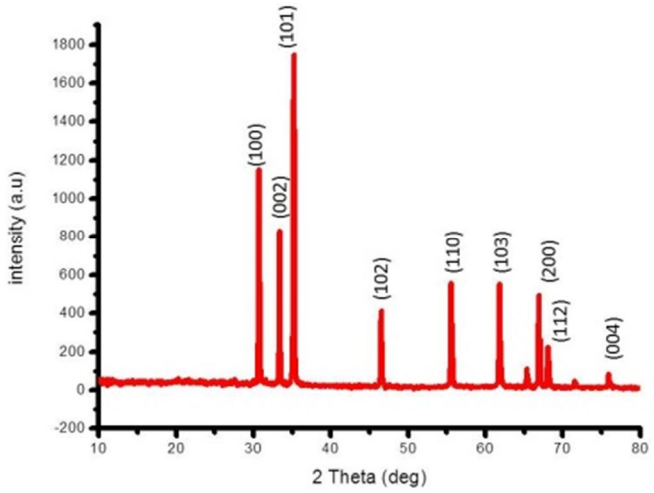

Figure.5 X-ray diffraction analysis

D. Field Emission Scanning Electron Microscopy and Energy Dispersive Spectroscopy

The morphology of the synthesized nanoparticles was examined by employing Field Emission Scanning Electron Microscopy of the Zinc oxide nanoparticles. The FESEM image exhibited that the formation of nanoparticles are almost spherical in shape under the diameter range of 50 nm as shown in Fig.6

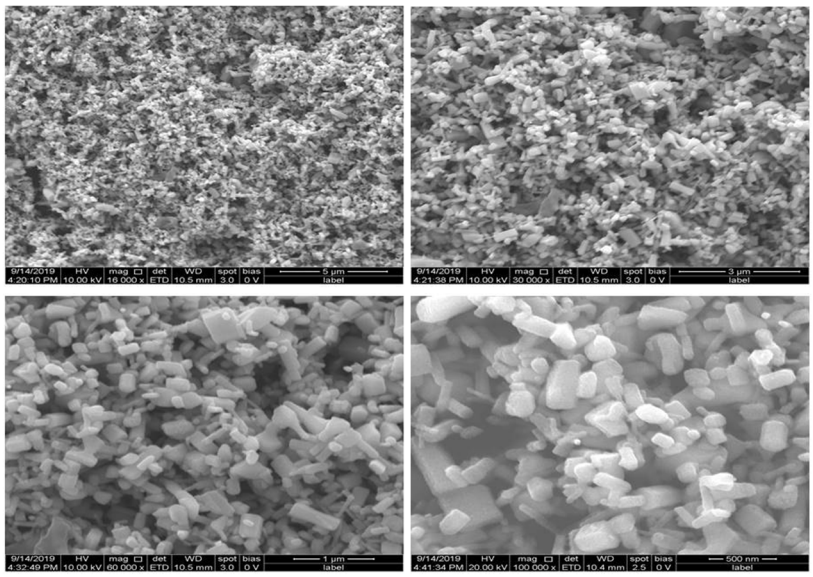

Figure.6 Field Emission Scanning Electron Microscopy

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The Energy Dispersive X-ray Diffractive (EDAX) study was conducted for the synthesized ZnO nanoparticles to make clear about the elemental composition. Also EDAX confirms the existence of Zinc oxide nanoparticles as illustrated in Fig.6 [15].

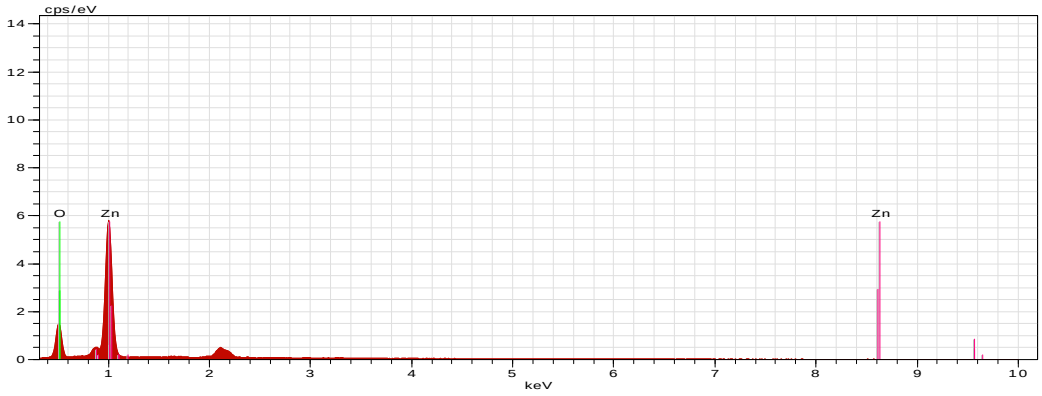

Figure. 6. Energy Dispersive Spectroscopy

E. Dynamic Light Scattering

The Fig.7 shows the size distribution images (DLS) of biosynthesized Zinc Oxide nanoparticles and the formation of ZnO nanoparticles. The resultant calculated average particle size distribution of this nanoparticles is 124 nm. The particle size of the synthesized Zinc oxide nanoparticles has confirmed [16].

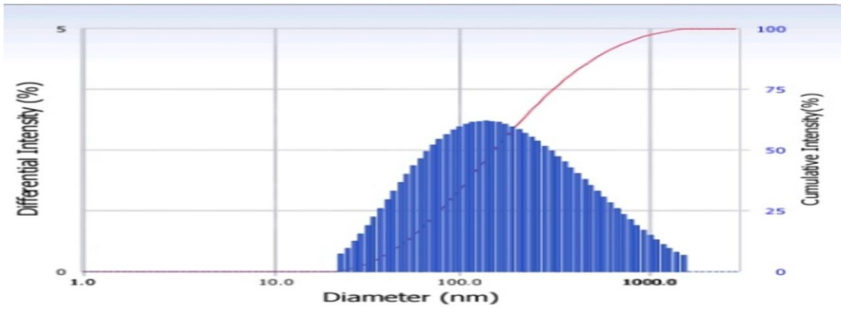

Figure.7 Dynamic Light Scattering

F. Comparative of Antibacterial and Antifungal Activities

The experiment represents the Antibacterial and Antifungal activities. By using disc diffusion method, the results of the antibacterial activity of different samples were tested against pathogens are shown in table.1. The inhibitory activity against two positive strains staphylococcus epidermidis (7 mm) and bacillus subtilis (3 mm) and one negative strains Klebsiella pneumonia (6 mm) were shown by sample D whereas at sample D exhibited the antibacterial activity in all bacteria. But as shown in Fig.8 sample C was more susceptible against Staphylococcus epidermidis (7 mm), Bacillus subtilis (3 mm) and Klebsiella pneumonia (6 mm).

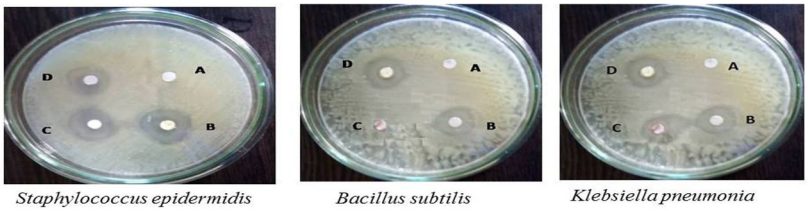

Figure.8 Antibacterial activity

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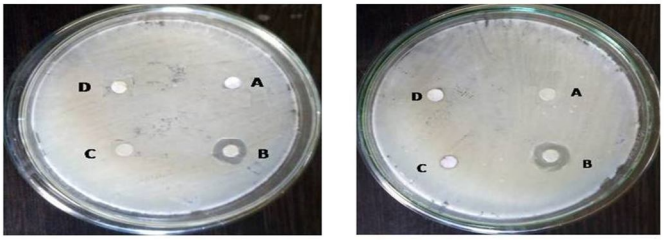
Table 1: Antibacterial activity of Zinc oxide nanoparticle sample

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)		
		Staphylococcus epidermidis	Bacillus subtilis	Klebsiella pneumonia
A (Zinc oxide)	10	0	0	0
B (Amoxicillin)	10	9	9	9
C (Plant extract)	10	5	0	4
D (Nanoparticles)	10	7	3	6

The results of the antifungal activities of different samples were tested against pathogens Candida albicans and Candida vulgaris are shown in table.2.



Candida albicans *Candida vulgaris*

Figure.9 Antifungal activity

Table 2: Antifungal activity of zinc nanoparticles

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)	
		Candida albicans	Candida vulgaris
A (zinc oxide)	10 µl	0	0
B (Fluconazole)	10 µl	8	8
C (Plant extract)	10 µl	0	0
D (Nanoparticles)	10 µl	0	0

V. CONCLUSION

The Zinc oxide (ZnO) nanoparticles was prepared by green method using Aristolochia bracteolata leaves extract as reducing agent. The pure ZnO crystalline structure has been confirmed by XRD Spectrum and on the other hand the final outcome of DLS and FESEM manifested the size of nanoparticles were in nanometer scale. In addition, the FESEM results confirmed spherical morphology and formed smaller nanoparticles at high temperature. FTIR spectroscopy was employed for the identification of the functional groups. The presence of ZnO nanoparticles was confirmed using UV-Visible spectroscopic studies were confirmed the observed absorption band. The both gram positive and gram negative bacteria such as Staphylococcus epidermis, Bacills subtilis and Klebsiella pneumonia. Two gram positive Staphylococcus epidermis, Bacills subtilis and One gram negative Klebsiella pneumonia were experienced to analyse the antibacterial activity of the ZnO nanoparticles. To analyse the antifungal activity of the ZnO nanoparticles with the help of fungi called Candida albicans and Candida vulgaris.

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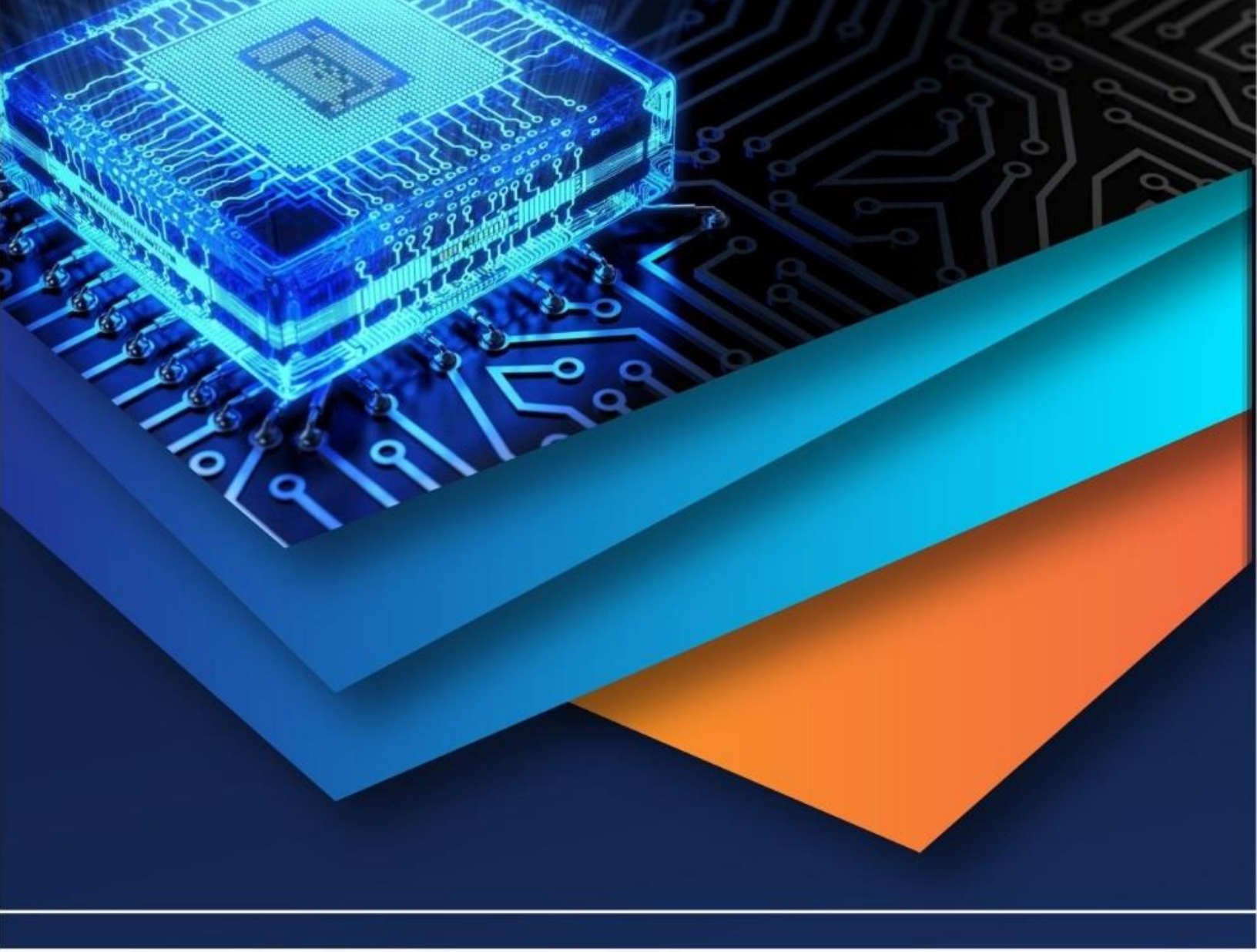

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