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# Bio Mediated Synthesis of Silver Nanoparticles using Moringa Oleifera Bark Extract and its Antimicrobial Activity against Human Pathogens

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***Abstract:*** ***There is an increasing commercial demand for nanoparticles due to their wide applicability areas such as electronics, chemistry, catalysis, energy and medicine. Metallic nanoparticles are traditionally synthesized by wet –chemical techniques, where the chemicals used are quit toxic and inflammable. In the present report, bio-reduction of silver nitrate into silver nanoparticles using the plant of Moringa oleifera barks is reported. The synthesized silver nanoparticles are characterized by XRD, FTIR, UV-visible and antimicrobial activity analysis. From the XRD analysis it is found that the synthesized silver nanoparticles were the Face Centered Cubic structure and the crystalline size of the sample is 22.08 nm. FTIR data proved that the components in Moringa oleifera barks act as good reductants and stabilizers for the silver nanoparticles. The absorption in the visible region and broad peak at 480 nm was studied by UV studies. The antimicrobial activity of bio-synthesized silver nanoparticles was analyzed against E.coli and Staphylococcus bacteria. The results revealed that the bark extract is a very good bio reductant for the synthesis of silver nanoparticles.***

***Keywords: Biosynthesis; Ag nanoparticles; XRD; FTIR; UV-visible; Antimicrobial activity;***

## I. INTRODUCTION

The numerous potential applications of nanoparticles have played a prominent role in the search for eco-friendly processes for generating nanoparticles using different biological materials because conventional nanoparticles syntheses involve the use of toxic solvents, high pressure, and high energy, all of which may be harmful to the environment. Such syntheses are capable of yielding nanoparticles that have unique attributes and properties that may influence their utility. Because of the rich biodiversity of microbes and plants,the potential for using biological materials in the synthesis of nanoparticles has yet to be fully explored. In this regard, several biological resources of the tropics, particularly those of Nigeria, have not been adequately evaluated for their potential use in synthesizing nanoparticles. The green synthesis of nanoparticles has continued to receive unprecedented attention due to the simplicity of the processes, the minimal chemical handling needed, and the eco-friendliness [1].In addition, the availability of several biological macromolecules/substances that can serve as capping and stabilization molecules for the green synthesis of nanoparticles has also contributed to the steady rise of this synthesis route. Various authors have reported using bacteria, fungi, algae, spider cobwebs and plant extracts for the green synthesis of different types of nanoparticles[1].

Moringa oleifera (Family: Moringaceae, English name: drumstick tree) has been reported to be essentially used as an ingredient of the Indian diet since ages. It is cultivated almost all over India and its leaves and fruits are traditionally used as vegetables. Almost all parts of the plant have been utilized in the traditional system of medicine. The plant leaves have also been reported for its antitumor, cardio protective, hypo tensive, wound and eye healing properties [2]. AgNPs synthesized from the aqueous extract of Moringa oleifera leaves in hot condition, have been reported in literature [3]. In the present study, synthesis of AgNPs in cold condition has been reported, reducing the silver ions present in the silver nitrate solution by the aqueous extract of Moringa oleifera leaves. Further, these biologically synthesized nanoparticles were found to be considerably sensitive to different pathogenic bacterial strains tested.

Metal nanoparticles and their alloys made of some combination of silver, iron, cadmium, zinc, platinum, gold, grapheme, among others have diverse applications in different aspects of human endeavours, with catalytic, optical, electronic, magnetic, antimicrobial and biomedical utility. Silver nanoparticles, for instance, are of interest because of their unique properties (size- and shape-dependent optical, electrical, and magnetic properties), which can be incorporated into coating materials, antimicrobial applications, biosensor materials, composite fibres, and electronic components [4].The aim of the current study was synthesis and optimization of AgNPs using bark extract of Moringa oleifera. Characterization of silver nanoparticles usingUV–Vis spectroscopy,

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XRD, FT-IR, and evaluation of the bactericidal activities against ,Escherichia coli and Staphylococcus to check their biomedical importance.

## II. MATERIALS AND METHODS

### A. *Collection of Plant Samples*

Moringa oleifera plants were collected from Tirunelveli District, Tamilnadu, India. The barks of the plant were thoroughly washed thrice with tap water and then with double distilled water to remove dust particles, air-dried for a week under shade at room temperature, finely cut, milled into a fine powder and was stored in airtight containers for later analysis.

### B. *Preparation of Aqueous Bark Extracts*

15 gm of the bark powder was mixed well with 100 ml of double-distilled water and boiled at 60ºC for 30 min. After boiling, the extract was filtered through Whatman No.1 filter paper. The supernatant was collected and stored at 4 ºC for further nanoparticles process.

### C. *Synthesis of Silver Nanoparticles*

1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 1 ml of M.oleifera bark extract was added into 100 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag+ ions and kept at room temperature for 24 hours. Formation of reddish brown colour confirmed the silver nanoparticles.

## III. CHARACTERIZATION STUDIES

The green synthesized silver nanoparticles were characterized by the following methods

### A. *UV Spectrophotometric Analysis*

The formations of leaf extract mediated silver nanoparticles were confirmed by the spectral analysis. The UV spectra of the biosynthesized silver nanoparticles were recorded using shimadzu UV-1800 Spectrophotometer by continuous scanning from 200nm to 900nm and distilled water was used as the reference for the baseline correction.

### B. *Fourier Transform Infra-Red SPECTROSCOPY Analysis*

The functional groups in the biosynthesized Ag NPS solution ere analyzed by FTIR spectroscopy. These measurements were carried using a perkin Elmer spectrum RX I FTIR instrument with a wavelength range of 4000 to 400 nm .The results were compared for shift in functional peaks.

### C. *XRD Analysis*

The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min. The purified Ag nanoparticles are dried. The structure and composition of Ag nanoparticles were studied by XRD (XPERT-PRO Machine). The data was collected in the 2Θ range. The crystalline domain size was calculated from the width of XRD peaks using Scherrer's equation.

Debye- Scherrer's equation, $D = K\lambda / \beta \cos \Theta$;

Where, D = average crystalline domain size;

β denotes Full Width at Half Maximum (FWHM),

K= 0.94,

λ = wave length of X ray,

And Θ is the diffraction angle.

### D. *Antibacterial Activity*

The antimicrobial activity of AgNPs was estimated against pathogenic bacteria such as Staphylococcus aureus (gram- positive bacteria's), E. coli (gramnegative bacteria's) by disc diffusion method[19].The bacterial cultures were grown in Brain Heart Infusion liquid medium at 37 °C. After 12 hrs of growth, each micro organism, at a concentration of 1 x 106 cells/mL equivalent to 0.5 Mc Farland Standard was spread on the surface of Mueller-Hinton agar plates. The dilutions were made in sterile low glucose Nutrient broth. Test pathogens were spread on the test plates- Muller Hinton agar for bacteria. The synthesized Ag nanoparticles and

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positive control were loaded onto 6 mm diameter sterile discs and placed in the plates respectively. After 24hrs of incubation, the zone of inhibition (mm in diameter) was measured and taken as the activity against the test pathogen.

## IV. RESULTS AND DISCUSSION

### *A. UV–Vis Spectral Studies*

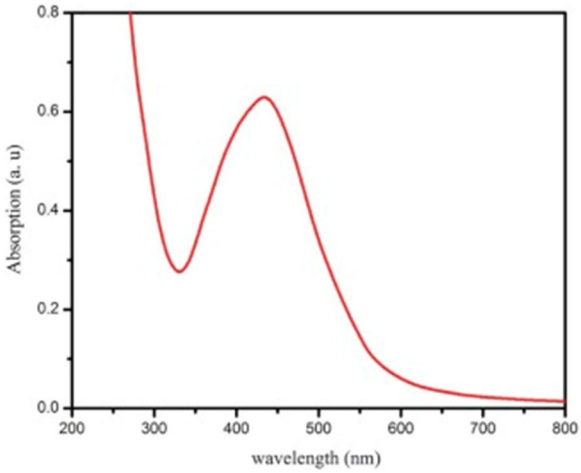


Figure1: UV-Vis spectrum of biosynthesized Ag nanoparticles.

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The plant extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from light green to dark brownish due to reduction of silver ion, which may be the indication of formation AgNP's .The UV- spectrum of Moringa oleifera Ag-Np's was recorded from the reaction medium. The results showed maximum absorption peak ranging between 390 – 410 nm

### *B. FTIR studies*

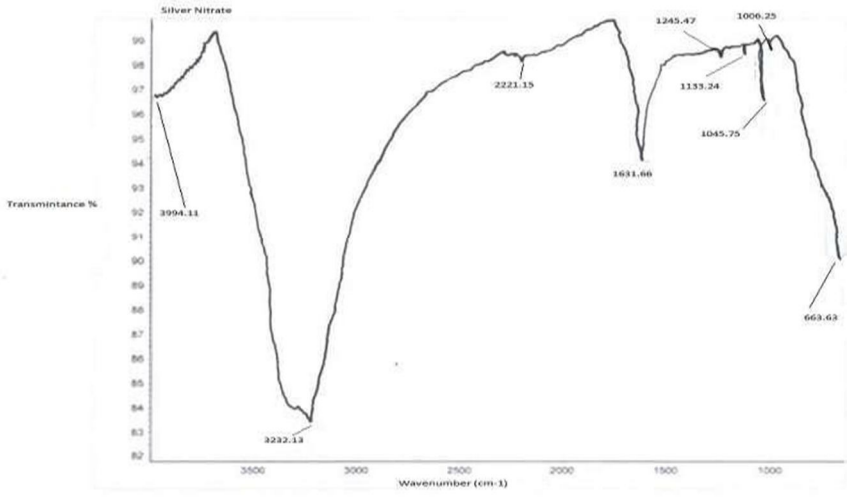


Figure 2: FTIR spectrum of Ag nanoparticles.

Fig. 2 showed intense FT-IR bands observed at 3232.14 and 1631.66 $cm^{-1}$ which indicate the presence of molecular functional groups that are responsible for the reduction of silver ions. The strong and broad peaks at3232.13 $cm^{-1}$ unveil the presence of phenolic compounds with hydroxyl group (663.63 $cm^{-1}$ ). This also might be ascribed to the hydrogen-bonded O-H stretch. Other

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than that, functional group of C=C stretching which is alkene existed in the silver nanoparticles based on the observable strong peak at 1631.66 $cm^{-1}$.In addition, this band might designated to the vibrational frequencies corresponding to amide protein. The absorption bands at the 2700 to 1850 $cm^{-1}$ region usually come only from triple bonds and other limited types of functional groups. Stretching band can be found in silver nanoparticles which are corresponding to aldehyde stretch groups. Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1245.47 $cm^{-1}$ and 1133.24 $cm^{-1}$. The C-O single bond can vary anywhere between 1000 cm−1 and 1300 cm−1 depending what sort of compound they are in. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles[6]. The results also entail that the phenolic compounds and protein may play a vital role in the formation of AgNP's. However, several reports have suggested that the formation of AgNPs might be due to the presence of proteins, free amine, carbonyl, and phenolic groups. This functional group may encapsulate the surface of silver ions by stabilizing the nanoparticles as capping agents. But the exact mechanism involved in the formation of AgNP's is still a debated.

### *C. Structural Studies*

X-ray diffraction (XRD) pattern of green synthesized Ag NPs was recorded in the 2θ range 20–80° shown in Fig.3. The XRD pattern shows the face center cubic structure of silver crystal, having diffraction peaks at 38, 44.3, 64, 42 and 77.2° correspond to (111), (200), (220) and (311) planes. The diffraction peak at 38° had a robust diffraction intensity indicating the preferential orientation of silver crystal along (111) plane. Non-indexed peak at 46.3° is the diffraction peak related to crystallization of bio-organic phase [5]. The highest peak intensity of (111) plane with narrow FWHM illustrates the good crystalline nature of synthesized Ag NPs as observed from the XRD images. The average crystallite size of Ag NPs is calculated from well-known Scherrer's formula [6].Synthesized Ag NPs of calculated mean crystallite size was 22.08 nm.

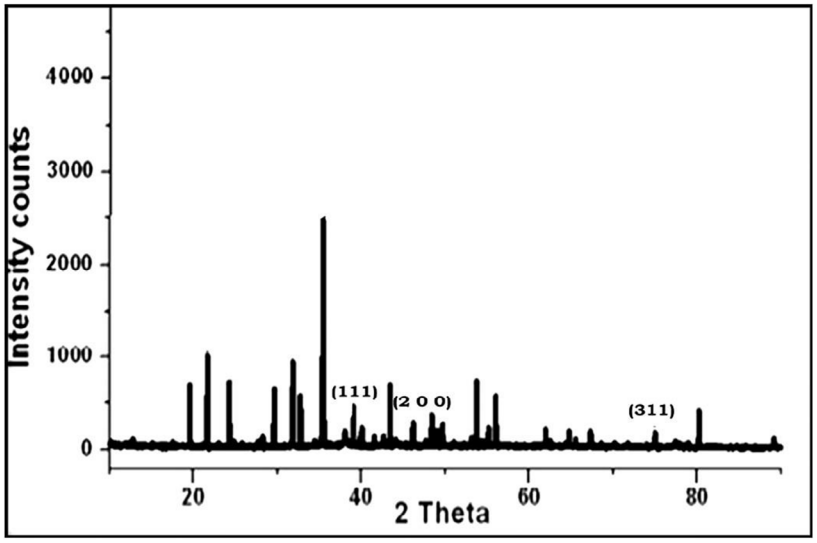


Figure 3: XRD pattern of Ag nanoparticles

### *D. Antimicrobial Activity*

Synthesized Ag NPs were examined about their antibacterial activity against the bacterial strains of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* using well diffusion technique. The zone of inhibition around the well is shown in figure 4. From Figure 4, it is noted that synthesized Ag NPs show significant impact on the growth of bacteria around the well. No inhibition zone was observed for control, prepared by the stack solution taken in well without Ag NPs. The antibacterial activity of Ag NPs increases gradually as the stack solution increases from 50 to 200 µL. The antibacterial activity of Ag NPs should be associated with several mechanisms including (i) generation of Reactive Oxygen Species (ROS) like super oxide anions ($O_2^-$) and hydroxyl radicals ($OH^\bullet$), (ii) the presence of $Ag^+$ ions in Ag NPs are making bond with sulphhydryl groups which direct to de-naturation of proteins in the bacteria and (iii) release of $Ag^+$ ions from the Ag NPs which simply penetrate into the cell wall and cause severe damage to the bacteria and kill them. Moreover, nanosized Ag NPs were attached to the bacteria and disturb the usual function of bacteria and hence damage severely to outer surface of the bacteria such as DNA, lipids and proteins. From Figure 4, it is found that Ag NPs have robust antibacterial activity on *E. coli* (Gram negative) than *S. aureus* (Gram positive) bacteria. This greater antibacterial

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activity against gram negative bacteria is ascribed to the variation in cell wall membrane of these bacteria. The gram negative bacteria such as *E. coli* consist of a very thin layer cell wall membrane, its thickness ranged 13 nm and made up of peptidoglycans and lipopolysaccharides. On the other hand, the gram positive *S. aureus* bacteria have a very thick cell wall membrane, its thickness ranged from 12 nm and made up of large number of mucopeptides, lipoteichoic and acids murein [7]. In addition, *S. aureus* has an antioxidant enzyme and shows a strong oxidant resistance [8,9].

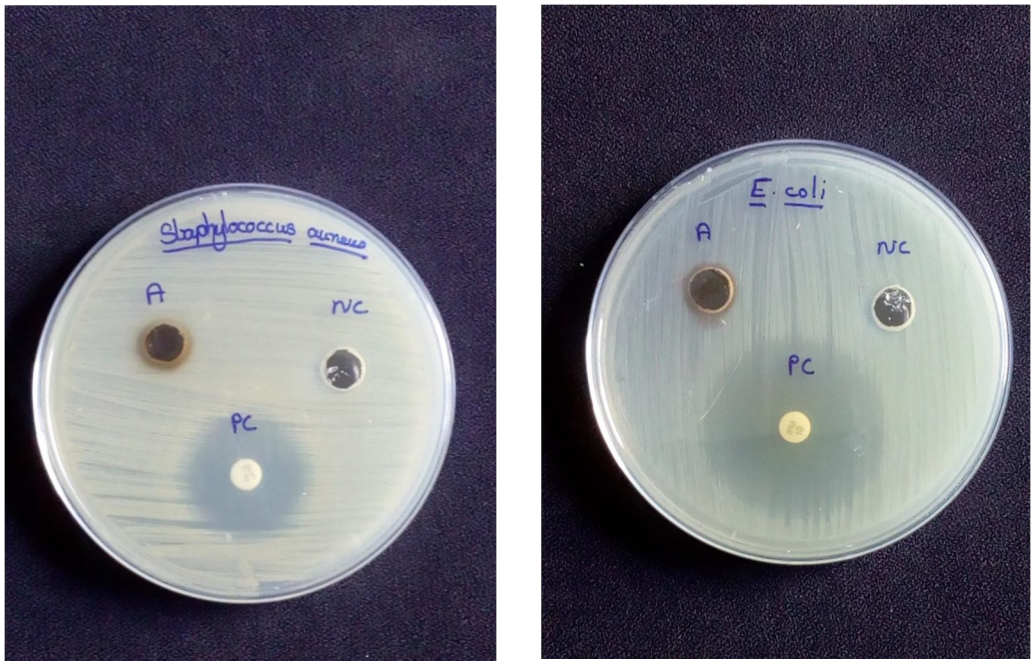


Figure 4:Antibacterial activity of Ag NP's against *E. coli* and *S. aureus* bacteria

## V. CONCLUSION

The rapid synthesis of stable silver nanoparticles of average size ~22 nm using Moringa oleifera bark extract was demonstrated. Achievement of such rapid time scales for synthesis of silver nanoparticles makes it more efficient as a biosynthetic pathway, though there still remains some scope for further decreasing the reduction time periods to make it a viable alternative to chemical synthesis methods. Probably the bio molecules responsible for the reduction and stabilization of AgNPs are phenols. The phenolics in Moringa oleifera exhibit excellent antioxidant activity and these phenols can react with a free radical to form the phenoxyl radicals. Therefore, the use of natural anti- microbial for the synthesis of AgNPs seems to be a good alternative which can be due to its benign composition. The plant material responsible for the reduction and stabilization of NPs needs further study including extraction and identification of the compounds presented in the extract.

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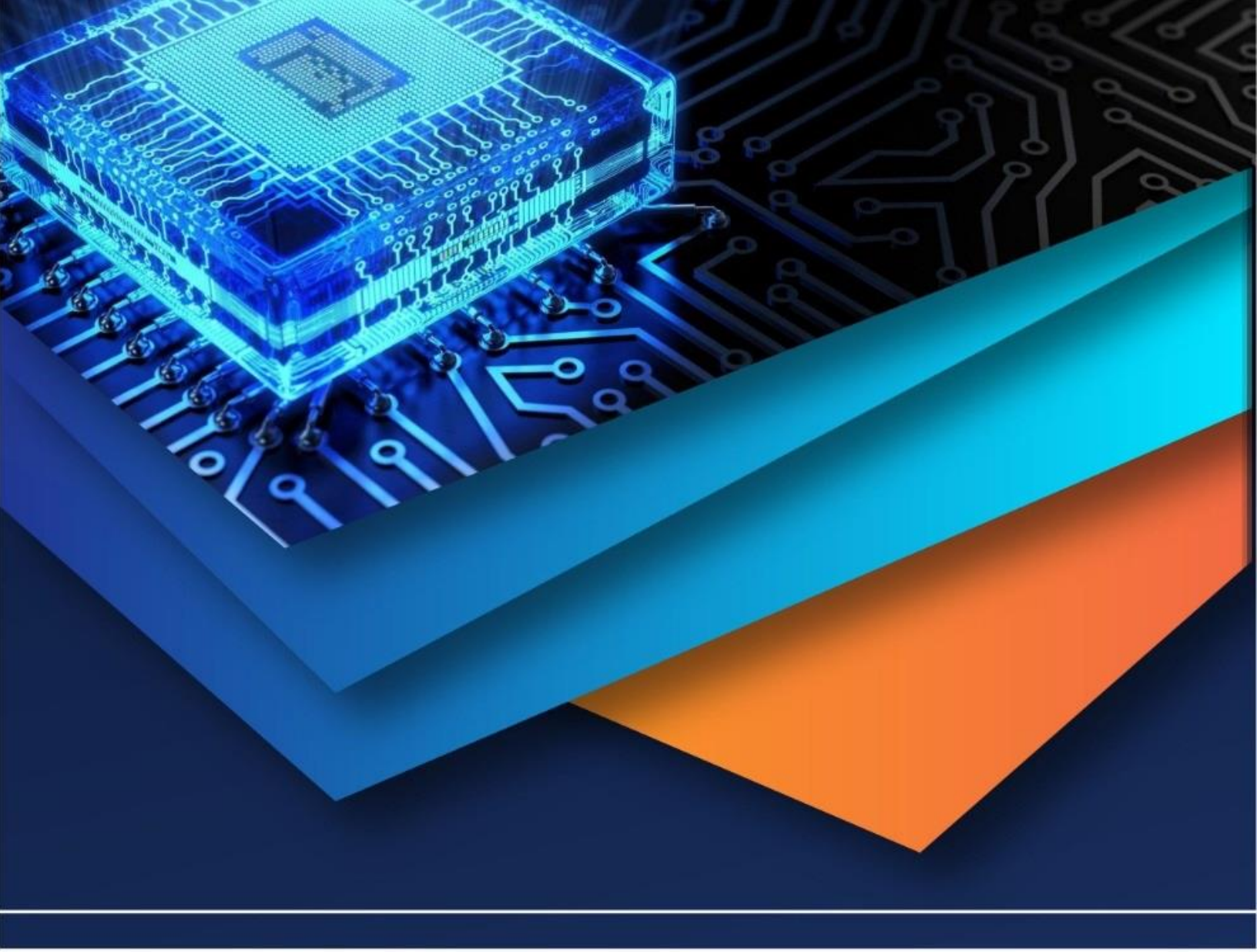

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