iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** III **Month of publication:** March 2018

DOI: http://doi.org/10.22214/ijraset.2018.3563

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue III, March 2018- Available at www.ijraset.com

Anti- Inflammatory And Anti- Bacterial Activity of Titanium Nanoparticles Synthesized From Rhizomes of Alpinia Calcarata.

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Abstract ***The present study was aimed to anti-inflammatory and anti-bacterial activities of titanium nanoparticles synthesized from Alpinia calcarata rhizomes of methanolic extract. Titanium nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in Titanium nanoparticles. The appearances of creamy colour in the reaction vessels suggest the formation of Titanium nanoparticles. UV-Visible and FTIR spectroscopy are further confirmed the structural characterization and functional group identification of titanium nanoparticles. The SEM analysis showed the particle size 60-130 nm as well the spherical structure of the nanoparticles. Titanium nanoparticles is useful for the development of newer and more potent antibacterial agents. The synthesized nanoparticle further confirmed its anti-inflammatory and anti-microbial activities. All the above data represented in the study contribute to a novel and unexplored area of nanomaterials as medicine.***
Keywords: Alpinia calcarata Rosec rhizomes, Titanium dioxide, Nanoparticles, Anti-microbial activity, Anti-inflammatory activity

I. INTRODUCTION

Nanotechnology is becoming a new area of increasing research and industrial interest since the 1980. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually working by the use of physical or chemical principles to develop a myriad of nano-objects with multiple applications.

Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others[1]. Nanochemistry or Nanotechnology are related with the production and the reactions of nanoparticles and their compounds.

It is concerned with the unique properties associated with assemblies of atoms or molecules on a scale between that of the individual building blocks and the bulk material (from 1 to 1000 nm).

This science use methodologies from the synthetic chemistry and the material's chemistry to obtain nanomaterials with specific sizes, shapes, surface properties, defects, self-assembly properties, designed to accomplish specific functions and uses. Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Bio-technology[2]. In recent years, titanium dioxide (TiO_2) has been extensively used as an environmentally harmonious and clean photocatalyst, because of its optical properties, high chemical stability and non-toxicity[3] . Titanium dioxide nanoparticles (TiO_2NPs) are one of the most important materials for cosmetics, pharmaceuticals[8], skin care, particularly to protect skin from UV rays, whiteness, opacity to products such as paints, plastics, papers, inks, food colorants and toothpastes[4]. In recent years, the biosynthetic method using plant extracts has received more attention than chemical and physical methods and even than the use of microbes, for the nano-scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. New strategies are therefore needed to identify and develop the next generation of drugs or agents to control bacterial infections. Earlier authors reported that the TiO_2 NPs were synthesized from Annona squamosa peel extract[5], Catharanthus rose us leaf aqueous extract[6] and Bacillus subtilis[7]. The present study aimed to Synthesis, characterise and evaluate the anti-inflammatory and antibacterial activities of titanium nanoparticles from Alpinia calcarata rhizomes extract.

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II. MATERIALS AND METHODS

A. Synthesis of Titanium nanoparticles (TiNPs)

Titanium nanoparticle synthesized by the method of Green synthesis of titanium dioxide nanoparticles[8]. Aqueous rhizomes extract of Alpinia calcarata was prepared using freshly grind rhizomes powder(20 g). The powder was boiled with 250 ml of double distilled water at 60 °C for 15 min. This extract was filtered through nylon mesh. For synthesis of Titanium dioxide(TiO_2) Nano-Particles, the Erlenmeyer flask containing 100 ml of TiO_2 (0.1 mM) was stirred for 2 hrs. 20ml of the aqueous extract of Alpinia calcarata was added in 80 ml of TiO_2 at room temperature under stirred condition for 24 hrs. The fresh TiO_2 solution and aqueous rhizomes extract of Alpinia calcarata didn't show any color change and there was no proof for the formation of nanoparticles. After the reaction of Alpinia calcarata rhizomes extract with TiO_2, the synthesized nanoparticles turned light brown in color.

B. UV-Visible Analysis

The extracts were examined under UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 330-830 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

C. FTIR- Spectroscopy

To determine Fourier transform infra-red (FTIR) pattern of the sample *Alpinia calcarata* filtrate containing the titanium nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FTIR Spectrum BX (Wellesley, MA, USA).

D. SEM analysis of Titanium Nanoparticle

Scanning Electron Microscopic (SEM) analysis was done using JSM 6701F – 6701 machine (Japan). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

E. In-Vitro Anti-Inflammatory Assay

In vitro anti-inflammatory activity was carried out by the method of Evaluation of in-vitro anti-inflammatory activity of TiNPs against the denaturation of protein[9].

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of Alpinia calcarata rhizomes extract and TiNPs (100, 200, 300, 400 and 500 µg/ ml respectively). Similar volume of double-distilled water served as control.

Then the mixtures were incubated at 37± 2°C in the incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using distilled water as blank. Diclofenac sodium at the final concentrations (100- 500µg/ ml) were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 x (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extracts concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

F. Anti-Bacterial Assay

Anti-bacterial assay was done by disc diffusion method[10]. Petri plates were prepared by pouring 30 ml of NA medium for bacteria The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 min. The surfaces of media were inoculated with bacteria from a broth culture.

A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar. Briefly, inoculums containing bacteria (*Escherichia coli* and *Staphylococcus aureus*) were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing 30µl of sample and 30µl for standard was laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24h for the bacteria. Each sample was tested in triplicate.

III. RESULTS AND DISCUSSION

A. *Synthesis of Titanium Nanoparticles*

The green synthesis of titanium dioxide nano particles through plant extracts were carried out. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous titanium ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of titanium hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the Alpinia calcarata rhizomes extract was responsible for the reduction of titanium ions. It is well known that titanium nanoparticles exhibit yellowish brown colour (Fig.1) in aqueous solution due to excitation of surface plasmon vibrations in titanium nanoparticles[11]. The appearances of yellowish-brown colour in the reaction vessels suggest the formation of titanium nanoparticles (TiNPs)[12].

Fig 1: Synthesis of titanium nanoparticles colour observation

B. *UV-Visible Spectroscopy*

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of titanium nanoparticles. It is quite sensitive to the presence of titanium colloids because these nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation. UV-Vis spectra from synthesized TiO_2 nanoparticles show the absorbance peak on 450nm[13]. With increasing particles size, the plasmon absorption shifts toward red. The adsorption spectra of the yellowish-brown TiNPs solution (Fig.2) prepared by phyto-reduction shows the surface plasmon resonance at about 330nm, indicating the presence of spherical and roughly spherical TiNPs. UV-Visible spectroscopy can be used as a characterization technique that provides information on whether the nanoparticle solution has destabilized over time. The optical properties of titanium nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighbouring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions. It is observed that the maximum absorbance of titanium nanoparticles occurs at 450nm. As the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates). The rapid and irreversible change in the extinction spectrum clearly demonstrates that the nanoparticles are agglomerating. In the present investigation the peak was decreased due to destabilization of nanoparticles.

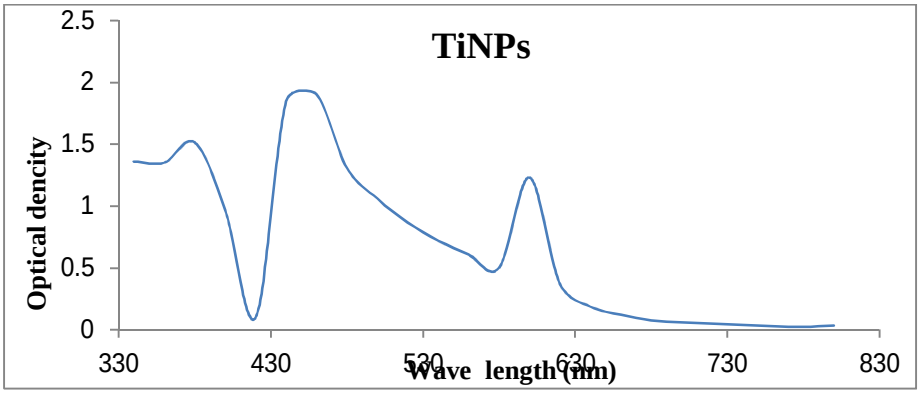


Fig 2: UV-VIS spectroscopy analysis TiNPs

C. FTIR Analysis

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. Fourier Transform Infrared (FTIR) spectroscopy can effectively be used to measure the particle formation. It is found that the width and intensity of peaks in an IR spectrum have explicit dependence on the particle size. As particle size increases, the width of the peak decreases and intensity increases[14]. The FT-IR spectra of TiO_2 nanoparticles as prepared and given in Fig.3. Many absorption bands belong to the organic groups such as OH and alkane were appeared. In TiO_2 as prepared sample, between 3800 to 3000cm^{-1} a broad band was observed which related to stretching hydroxyl(O-H), representing the water as moisture. The other peaks at 1648 cm^{-1} were indicated to stretching of titanium carboxilate, which formed from plant extract as precursors. The peak between 800 and 400cm^{-1} was assigned to the Ti-O stretching bands.

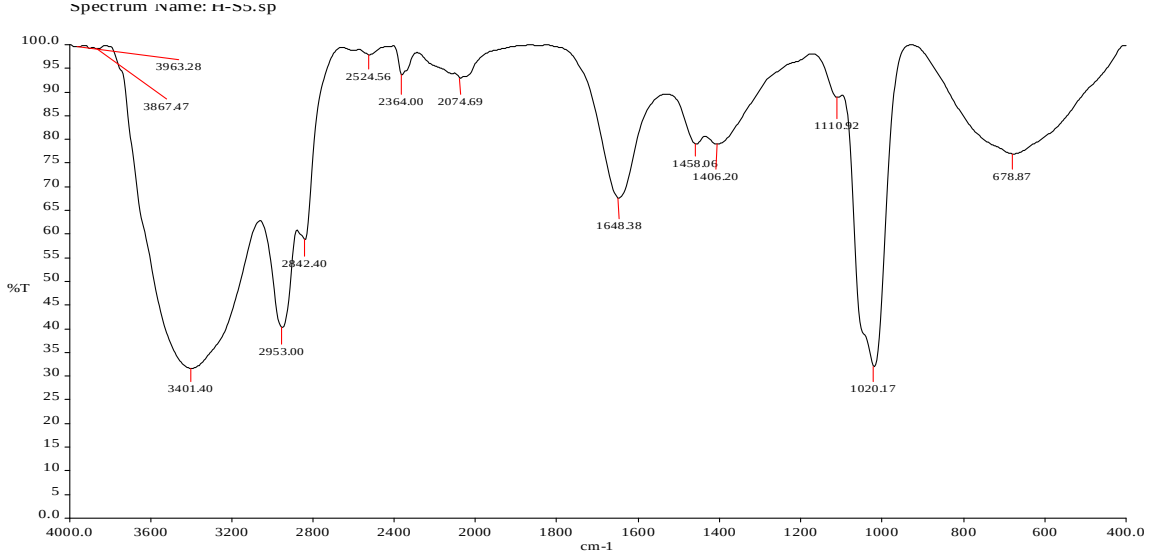


Fig 3: FTIR Analysis of titanium nanoparticles

D. SEM analysis

SEM analysis was carried out to understand the topology and the size of the TiNPs, which showed the synthesis of higher density polydispersed spherical TiNPs of various sizes. The SEM image showing the high density titanium nanoparticles synthesized by the Alpinia calcarta rhizomes extract and confirmed the development of titanium nanoparticles. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis of Alpinia calcarata rhizomes synthesized titanium nanoparticle showed the particle size between 60–130 nm as well as the spherical structure of the nanoparticles (Fig 4).

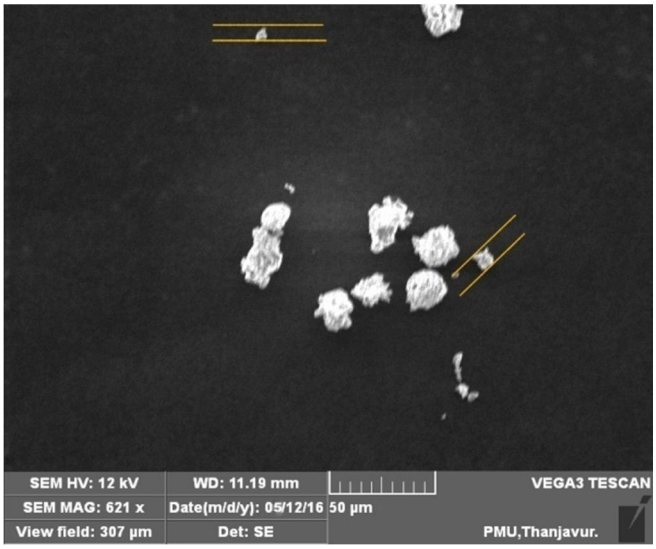


Fig 4: SEM Analysis of titanium nanoparticles

E. In vitro anti-inflammatory assay

There are certain problems in using animals for experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, for the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property *Alpinia calcarata* rhizomes. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding[16] . Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & bovine albumin) denaturation by and reference diclofenac sodium [17]. *Alpinia calcarata* rhizomes exhibited anti-inflammatory activities in dose dependent manner.

TABLE 1

In vitro anti-inflammatory activity of Alpinia calcarata rhizomes and titanium nano particles (Egg albumin)

S.No	Doses (µg/ml)	Plant extract	Titanium nano particles	Standard (Diclofenac sodium)
1	100	18.42±1.28	21.05±1.47	24.21±1.69
2	200	32.89±2.30	43.42±3.03	46.24±3.23
3	300	57.89±4.05	63.15±4.42	67.48±4.72
4	400	69.73±4.88	78.94±5.52	81.04±5.67
5	500	81.57±5.70	89.47±6.26	94.21±6.59

Values are expressed as Mean ± SD for triplicates

F. Antibacterial Assay

The efficacy of the Ti nanoparticles against *Escherichia coli* and *Staphylococcus aureus* may derive from the difference as a point of membrane structure. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ti nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ti nanoparticles as an antibacterial agent. The TiNPs synthesized from Alpinia calcarata species are toxic to multi-drug resistant microorganisms. The antibacterial activity of TiNPs near to the standard. It shows that they have great potential in biomedical applications against infectious diseases.

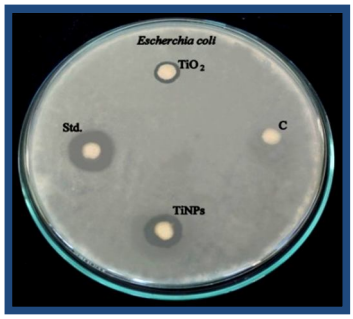
TABLE 2:

Antimicrobial activity of TiNPs, TiO_2 and Control

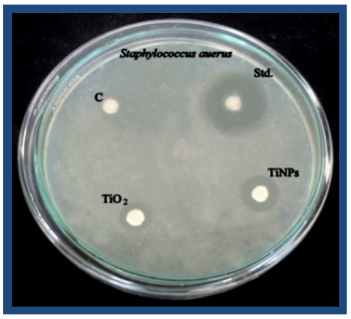
Microorganism (mm)	Titanium dioxide (30µl)	Titanium dioxide nanoparticles (30µl)	Standard (Chloromphenical) (30µl)	Control (Distilled water - 30µl)
Escherchia coli	1.58±0.11	3.94±0.27	6.87±0.48	0
Staphylococcus auerus	1.41±0.09	3.83±0.26	7.14±0.49	0

Values were expressed as Mean ± SD

Escherchia coli



Staphylococcus auerus



IV CONCLUSION

Alpinia Calcarata rhizome methanolic extract was used as a reducing agent for the green synthesis of TiNPs. The formation of TiNPs was confirmed by UV-Visible and FTIR spectroscopy. Their size (60-130nm) and shape were confirmed by SEM analysis. The synthesized TiNPs shows *in-vitro* anti-inflammatory activity and anti-bacterial activity. The results promised TiNPs are highly active than plant extracts.

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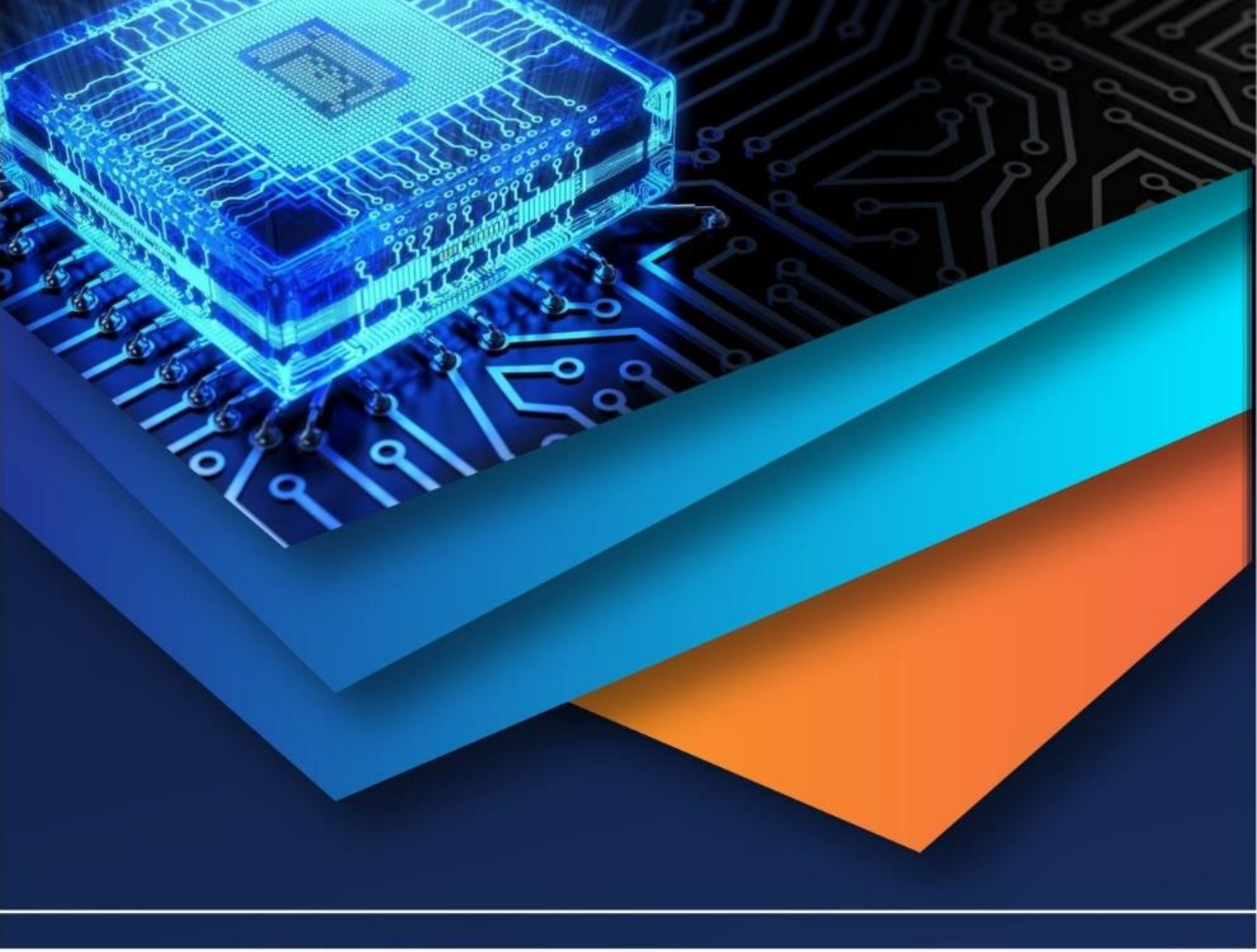

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