iJRASET
International Journal For Research in Applied Science and Engineering Technology

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

**Volume:** 6 **Issue:** IV **Month of publication:** April 2018

**DOI:** http://doi.org/10.22214/ijraset.2018.4502

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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 IV, April 2018- Available at www.ijraset.com*

# Antioxidant, Free Radical Scavenging and Antibacterial Potential of Biosynthesized Zinc Oxide Nanoparticles of Carica Papaya Seeds

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***Abstract:*** ***Introduction: Green synthesis of zinc oxide nanoparticles (ZnO NPs) is ecofriendly, inexpensive and nontoxic when compared to physical and chemical methods. In this paper, we report the synthesis and characterization of ZnO NPs using Carica papaya seeds extract.***
***Methodology: Preliminary phytochemical analysis were carried out using standard procedures with different solvents like chloroform, acetone, ethanol and aqueous. The enzymic and nonenzymic antioxidants present in Carica papaya seeds were determined. Free radical scavenging potential of ZnO NPs was assessed by various methods namely DPPH, hydroxyl radical, hydrogen peroxide, superoxide and nitric oxide scavenging assays. Moreover, total antioxidant activity was evaluated by phosphomolybdenum method. The antibacterial activity against the Carica papaya seed extract was also determined by agar well diffusion method. Result: Among the different extracts used, aqueous extract showed more positive results and hence it was used for further studies. The maximum absorbance of ZnO NPs was noticed at 274 nm in the UV-Visible absorption spectrum. The free radical scavenging activity of biosynthesized ZnO NPs was increased in dose dependent manner. The maximum zone of inhibiton was noticed in Escherichia coli.***
***Conclusion: The results revealed that zinc oxide nanoparticles of Carica papaya seed extract possess potent antioxidant and antibacterial properties.***
***Keywords: Zinc oxide nanoparticles, Carica papaya, UV-Visible spectroscopy, Escherichia coli.***

## I. INTRODUCTION

Nanotechnology is recent emerging technology which provides solutions to various fields in science. Nanoparticles are varied by altering their atomic and molecular properties. $10^{-9}$ is referred as a nanosize. “Green synthesis” is a clean, safe, eco-friendly and environmentally nontoxic method of nanoparticle synthesis. Among metal oxide nanoparticles, zinc oxide (ZnO) has received much attention in the recent past. ZnO nanostructures are the forefront of research due to their unique properties and wide applications [1]. Carica papaya belongs to the family of Caricaceae. They are used throughout Asia. The plant can be monoecious, diecious or hermaphroditic. Antioxidant activity is closely related to phenolic content of plants. Because synthetic antioxidant such as butylated hydroxytoluene or butylated hydroxanysol could promote cancer development in rats and the fact that consumers are much interested in natural food additives, herbal phenolic compounds and other natural antioxidants are extremely desirable. It has been observed that the fruit and seed extracts of papaya have antibacterial activity against Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Shigella Flexneri [2].

## II. MATERIALS AND METHODS

### A. *Collection of seeds*

The seeds of Carica papaya were taken for this study. Papaya was obtained from fruit vendors in the district of Coimbatore, Tamil Nadu, India. Seeds were shade dried and powdered using a mechanical blender.

### B. *Preparation of Solvent*

The powdered material was extracted with aqueous solution and placed in a shaker overnight. Crude obtained was further used in the production of zinc oxide nanoparticles (ZnO NPs) and to estimate the free radical scavenging efficacy.

### C. *Preliminary Phytochemical Analysis*

*The Carica papaya seed extract was qualitatively and quantitatively analyzed using standard procedures [3].*

*D. Green Synthesis of ZnO Nanoparticles*

To 50ml of distilled water, 0.2g of dehydrated zinc acetate was added. Sample solution was taken and heated at 50°C in a water bath. Zinc acetate solution was added drop-wise to it. After continuous stirring, add 2.0M NaOH till it reaches pH 12. The white precipitate was filtered off and washed thoroughly with distilled water. This was followed by ethanol wash and dried at 60 °C under vacuum oven overnight [4].

*E. Characterization of ZnO Nanoparticles*

ZnO nanoparticles synthesized were characterized by UV-Visible spectroscopy [5]. It can be read at 600nm.

*F. Glutathione Reductase*

To 1 ml of 0.12M Potassium phosphate buffer, 0.1 ml of EDTA, 10mM sodium azide, 6.3mM oxidized glutathione and sample is added. The contents are made up to 2 ml with distilled water. It is incubated at room temperature for 3 minutes. Absorbance is measured at 340nm at interval of 15 seconds. The control tube contains distilled water instead of glutathione. Enzyme activity is expressed as moles of NADPH oxidized.

*G. Glutathione – S- Transferase*

ml of substrate is added with 2.7 ml of phosphate buffer (pH 6.5), 0.1 ml of sample and made up to 3 ml with distilled water. Enzyme activity was determined by monitoring the change in absorbance at 340nm.

*H. Peroxidase*

To 0.02 ml of the sample 3 ml of 0.05M pyrogallol solution is added. Add 0.5 ml of 1% $H_2O_2$ and read at 430nm for every 30 seconds up to 3 minutes.

*I. Non-enzymic Antioxidants [7]*

1) *α-Tocopherol:* 1.5 ml of sample and 1.5 ml of standard, 1.5 ml of xylene is added. To the test 1.5 ml of ethanol is added whereas to the standard 1.5 ml of water is added. They are mixed well and centrifuged. To 1 ml of the supernatant 2ml of dipyridyl reagent is added to both the tubes. 1.5 ml of the solution is taken and read at 460nm. Then added 0.3 ml of ferric chloride and incubated at room temperature for 15 minutes and read at 520nm.

*J. Ascorbic Acid*

To 1ml of sample 1ml of ice cold 10% TCA is added and centrifuged for 20 minutes at 3500nm. From this 0.5 ml of supernatant is taken and 0.1 ml of DTC reagent is added. It is incubated at 37°C for 3 hours. To this 0.75 ml of sulphuric acid is added. The tube is incubated at room temperature for 30 minutes and read at 540nm. Standard ascorbic acid is taken in the concentration of 10-50µg.

*K. Reduced Glutathione*

Reduced Glutathione reacts with DTNB to give yellow coloured product which can be measured at 412nm. To 0.6ml of sample 1.0 ml of Ellman's reagent is added and 3.0 ml of $Na_2HPO_4$ is added. The colour is read at 412 nm.

*L. Free Radical Scavenging Activity*

1) *DPPH spectrophotometric activity [8]:* The sample extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Ethanol served as the blank and DPPH in methanol, without the seed extracts, served as the positive control. After 30 minutes of incubation, the discoloration of the purple color was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = \frac{\text{A518 (sample)} - \text{A518 (blank)}}{\text{A518 (blank)}}$$

*M. Hydroxyl Radical Scavenging Activity [9]*

0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of $H_2O_2$, 0.1ml of ascorbate, 0.1ml of $KH_2PO_4$-KOH buffer and 20µl of plant extracts was made upto a final volume of 1.0ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 minutes to develop the color. After cooling, the TBARS formation was measured spectrophotometrically at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined

by comparing the absorbance of the control with that of the samples. The percent TBARS production for positive control ($H_2O_2$) was fixed at 100% and the relative per cent TBARS was calculated for the extract treated groups.

*N. Hydrogen Peroxide Scavenging Activity*

A solution of $H_2O_2$ (40mM) was prepared in phosphate buffer. Seed extracts at the concentration of 10mg/10µl were added to $H_2O_2$ solution (0.6ml) and the total volume was made up to 3ml. The absorbance of was read at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without $H_2O_2$ was prepared.

*O. Superoxide Scavenging Activity* [10]

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the seed extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

*P. Nitric oxide SCAVENGING activity* [11]

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of seed extracts (50mg) and incubated at 25°C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the extracts. The absorbance was read at 546nm in a spectrophotometer.

## III. RESULTS AND DISCUSSIONS

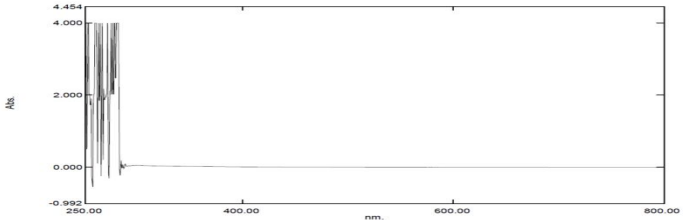
Preliminary phytochemical screening of various extracts of Carica papaya seeds showed the presence of active phytochemical constituents like Saponins, Tannins, Flavonoids, Carbohydrates, Proteins, Phenols and Alkaloids. The aqueous extract of Carica papaya proved to have more rich phytoconstituents when compared to other extracts (Hydroethanol, Water, Chloroform and Acetone) on par with Kaibing Zhou et al., 2011.

Table 1: Preliminary Phytochemical Screening

| Extract | 50%Ethanol | Acetone | Petroleum ether | Water | Chloroform |
|---|---|---|---|---|---|
| Saponins | - | + | - | + | - |
| Tannins | + | + | - | + | + |
| Flavonoids | - | + | - | + | + |
| Carbohydrates | + | - | - | + | - |
| Proteins | - | - | + | + | - |
| Phenols | + | + | - | + | - |
| Alkaloids | + | + | + | + | - |

UV-visible absorption spectroscopy is a widely used technique to examine the properties of nanosized particles. UV spectroscopy result is depicted in figure (1). The absorbance maximum is seen at 274nm confirming the formation of zinc oxide nanoparticles. Manokari, *et al*., 2016 also studied the UV-Visible recording for leaves, flowers and fruits that falls at 296 nm.

Fig 1 UV Visible spectral analysis of ZnO nanoparticles



papaya contains proteins (0.56mg/g), tannins (0.36mg/g), phenols (0.46mg/g) and flavonoids (0.46mg/g). Due to the high protein content of the Carica papaya seeds exhibits effective antioxidant activity which are related to the work done by Mittal et al., 2016 and Asmah et al., 2013.

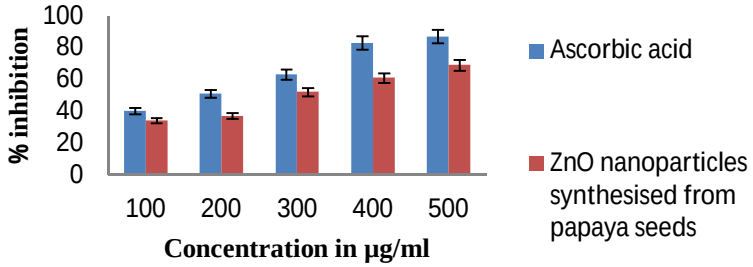
The presence of enzymic and nonenzymic antioxidants in the ZnO nanoparticles have paved way for their use in medical field to treat and cure illness. Thus the seeds can be used for their medicinal properties with suitable extract such as water.

Table 2: Enzymic and non enzymic antioxidants

| ENZYMIC ANTIOXIDANTS (mg/g) | | | | |
|---|---|---|---|---|
| *Carica papaya* seeds | Superoxide dismutase | Glutathione reductase | Glutathione-s-transferase | Peroxidase |
| Water | 66.55±3.1 | 3.4±0.29 | 20.22±0.61 | 52.1±0.84 |
| NONENZYMIC ANTIOXIDANTS (mg/g) | | | | |
| *Carica papaya* seeds | Carotenoids | Ascorbic acid | α-tocopherol | Reduced glutathione |
| Water | 0.569±0.04 | 0.195±0.005 | 0.702±0.16 | 0.594±0.11 |

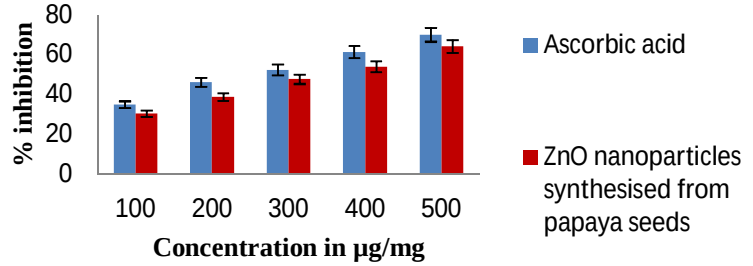
The percentage of free radical scavenging activity was determined against DPPH and it is depicted in figure (2). Zinc oxide nanoparticles of Carica papaya show 75% of inhibition at 500 µg/ml when compared with standard ascorbate. The $IC_{50}$ value was found to be 24.8±3.9µg/ml.

Fig 2 DPPH scavenging activity



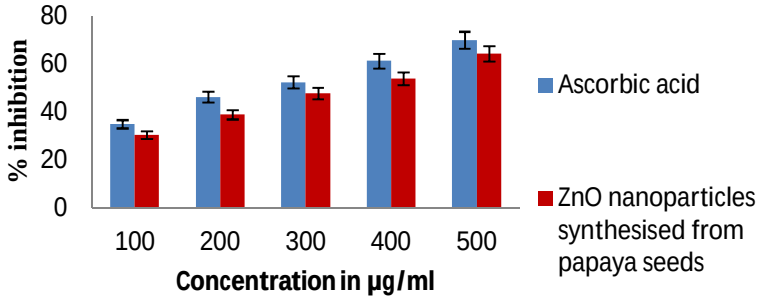

The superoxide radical inhibition is shown in the figure (3). It shows a moderate increase in the percentage of inhibition with increase in the concentration. The zinc oxide nanoparticles show 62% inhibition of SO radical at 500µg/ml concentration with respect to standard ascorbate. The $IC_{50}$ was found to be 34.29±2.81µg/ml. Mittal *et al.*, 2016 reported that the scavenging activity is due to the presence of water soluble compounds like phenol and flavonoids.

Fig 3 Inhibition of superoxide radical



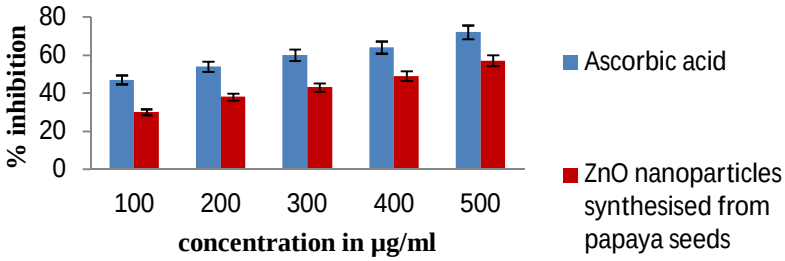
The scavenging activity may help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health (Moncada and Higgs, 2006. Inhibition of NO radical is also a measure of antioxidant activity. It is depicted in figure (4). At the concentration of 500µg/ml the zinc oxide nanoparticles has shown 69.98% of inhibition of nitric oxide. The $IC_{50}$ value was found to be 42.57±1.26µg/ml. The results are in relation with the results reported by Saranya and Shanthi, 2016.

Fig 4 Inhibition of nitric oxide radical



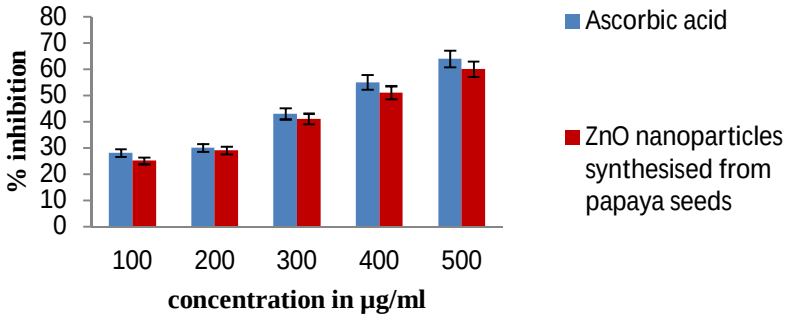

Inhibition of hydroxyl radical by the zinc oxide nanoparticles is shown in the figure (5). From the figure it is clear that at higher concentration the % inhibition of the hydroxyl radical is high. The results are similar to the results reported by Saranya and Shanthi, 2016. The percentage of inhibition at 500µg/ml is 35%. The $IC_{50}$ value was found to be 39.9±7.96µg/ml.

Fig 5 Inhibition by hydroxyl radical



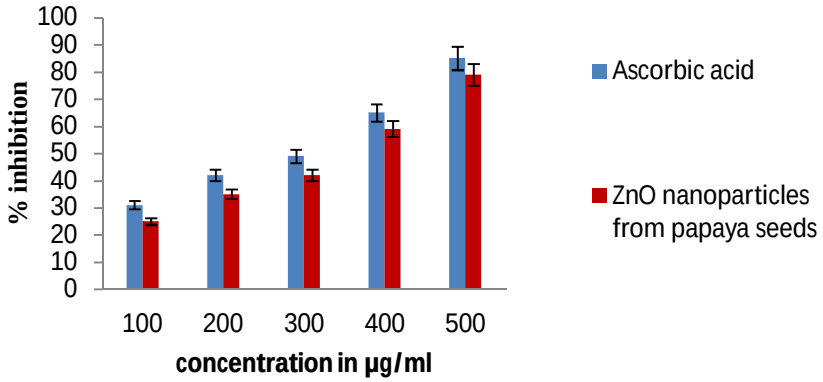

Hydrogen peroxide is a weak oxidizing agent and can directly inactivate few enzymes usually by oxidation of essential thiol (-SH) groups. The present results showed that papaya seeds have low to high $H_2O_2$ scavenging ability and observed to be in a concentration dependent fashion. It is depicted in figure (6). At maximum concentration, the inhibition is 59.29%. The $IC_{50}$ value was found to be 33.2±2.7µg/ml.

Fig 6 Inhibition by hydrogen peroxide



The total antioxidant capacity was evaluated by phosphomolybdenum method. This method is used to estimate the total antioxidant in the sample. The result of Phosphomolybdenum assay is given in the below figure (7). The reducing capacity depends on the phenolic contents. 78.14 % of inhibition is seen by the ZnO nanoparticles at the concentration of 500µg/ml. The $IC_{50}$ value was found to be 33.42±7.0µg/ml. The result is in accordance with the result stated by Phatak and Hendre, 2014.

Fig 7 Phosphomolybdenum assay




In the antibacterial activity, it was studied that the Zinc oxide nanoparticles are more efficient than the crude aqueous extract of Carica papaya seeds. The MIC values were found to be 5µg/ml for S. aureus and 10µg/ml for E. coli. Thus ZnO nanoparticles enhances the antibacterial property of the seeds (Sirelkhatim et al., 2015).

Table 3: Zone of inhibition and MIC values

| S.NO | BACTERIA | ZONE OF INHIBITION *(Streptomycin)* (mm) | ZONE OF INHIBITION (mm) | | MIC VALUES (µg/ml) |
|---|---|---|---|---|---|
| | | | CRUDE | NANOPARTICLE | |
| 1 | *E. coli* | 13 | 0.7 | 3 | 10 |
| 2 | *S. aureus* | 10 | 0.3 | 1.2 | 5 |

## IV. CONCLUSION

Nanoparticles have great efficiency in reaching the target system. Thus zinc oxide nanoparticles are synthesized by green method and characterized using UV-Visible spectroscopy. The UV results revealed maximum absorbance of zinc oxide nanoparticles at 274nm.The presence of enzymic and non enzymic antioxidants in the ZnO nanoparticles have paved way for their use in medical field to treat and cure illness. The free radical scavenging potentials revealed that ZnO nanoparticles of Carica papaya are a strong antioxidant. The results of antibacterial activity showed that the seeds of Carica papaya have considerable inhibitory effect on bacteria namely E. coli and S. aureus. Thus the seeds can be used for their medicinal properties with suitable extract.

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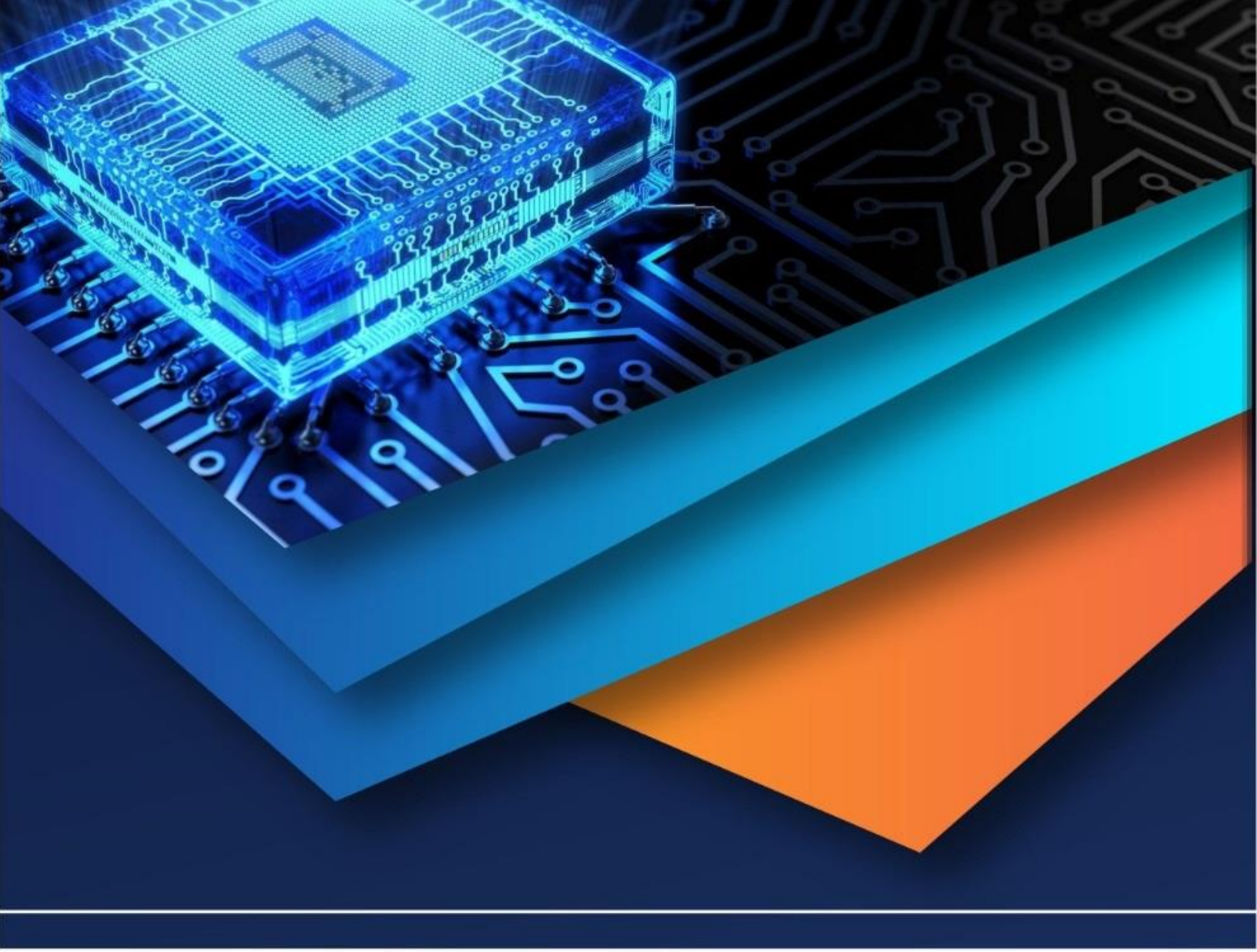

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