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Invitro Assessment of Antibacterial, Antioxidant and Drug Release Property of Extracellularly Bio-Fabricated Gold Nanoparticles using Pimpinella Anisum

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Abstract: Biosynthesis of nanoparticles is gaining more importance nowadays in research field. Especially using plant extracts, according to WHO about 80% of world population is belongs to plant/ herbal based medicine. Gold nanoparticles has wide range of biomedical applications such as drug delivery, cancer treatment, radiation therapy etc,. In the present investigation, the gold nanoparticles were synthesized using aqueous extract of Pimpinella anisum. The synthesised gold nanoparticles were characterized using UV- Visible spectrometer, SEM analysis, XRD analysis and FT-IR analysis. Further, the antibacterial activit y, antioxidant property and in vitro drug release assessment for the synthesised gold nanoparticles were performed.
Keywords: Gold Nanoparticles, Biosynthesis, Pimpinella anisum, Biomedical applications

I. INTRODUCTION

Nanotechnology is the day-to-day growing technology in modern research field [1]. Nanoparticles are 1000 times smaller than the human cells. Due to its small size and large surface area to volume ratio [2], it was utilized in drug delivery, bio- imaging, gene therapy, cancer therapy and bio molecules carrier etc [5]. Though the nanoparticles can be synthesized by an array of physical and chemical methods which cause disturbance in ecological balance because of toxic gas exhaust and requirement of high temperature, pressure etc [3]. Biosynthesis of nanoparticles is attaining a broad mind of research to synthesis metal nanoparticles using herbs/ medicinal plants. It is alternate to overcome the limitations of conventional method with low cost, simple, less time consumption and relatively reproducible method [6]. Gold nanoparticles are gaining a importance due to its biocompatibility and non- toxicity, which is used by human history since last 50 years [7]. A gold nanoparticle produces wide range of applications due to its optical property of SPR band formation from UV- Visible to IR region [4]. From the literature survey, it is novel to synthesise gold nanoparticles using *Pimpinella anisum*. *Pimpinella anisum* belongs to *Apiaceae* family, flowering plant commonly known as anise seed, one of the spices [8]. In ancient days, it is utilized for stomach disorders and commonly named as Shombu in Tamil. It is used as folklore medicine in human history as medicinal appliances.

II. EXPERIMENTAL SECTION

A. Chemicals

The 99.9% pure Chloroauric acid was purchased from Loba Chem Pvt Ltd, Chennai. The Deionised water, Ascorbic acid and Muller Hinton Agar medium were purchased and chemicals prepared were standard and merck.

B. Collection Of Plant

The anise seeds were purchased from shop in Kanchipuram and sowed in the agriculture field after 30 days, the plant were grown and matured after 65 days. The leaf of the plant were collected and washed with tap water to remove sand particles and dust, followed by distilled water.

C. Extract preparation

5g of fresh leaves were chopped into small pieces using sterile knife and taken in a 250mL flask containing 50mL of deionised water. The flask was kept onto the heating mantel for boiling at 80ºC for 20 minutes. After boiling, the flask were taken and allowed to cool. After cooling, the extracts were filtered using Whatmann filter paper No: 1. The collected filterate were stored at frozen condition for further research.

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D. Biosynthesis Of Gold Nanoparticles

1mM concentration of Chloroauric acid solution was prepared using deionised water. 5mL of plant extract was added to 95 mL of 1mM of Chloroauric acid solution and kept undisturbed condition at room temperature. After 1 hour, the color of the solution mixture was changed into ruby red was observed and the color change indicates the reduction of gold ions to gold nanoparticles formation. Further it was confirmed through characterization of colloidal solution using UV- Visible spectrophotometer, in the range of 400- 800nm.

E. Purification And Characterization Of Gold Nanoparticles

The colloidal solution was centrifuged at 10000 rpm and the supernatant were discarded. The pellets were dried at 60ºC in hot air oven and powder form of gold nanoparticles was collected. The powder forms of gold nanoparticles were subjected to SEM, XRD and FT-IR analysis.

F. Antioxidant Study

Hydrogen peroxide scavenging assay is used to study the antioxidant property of the synthesised gold nanoparticles. Different concentrations of synthesized gold nanoparticles were added to PBS buffer of pH 7.4 containing 40mM of H_2O_2 solution mixture. The solution mixture is kept for incubation at room temperature for 10 minutes and analysed at 560nm using UV- Visible spectrophotometer. The PBS buffer without H_2O_2 is used as blank. The ascorbic acid is used as standard. The percentage of inhibition was calculated from the absorbance values using the formula as follow as:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance Control}}{\text{Absorbance Control}} \times 100$$

G. Antibacterial Study

To analyse the antibacterial activity of the synthesized gold nanoparticles against One day culture of *E. coli* and *B. substilus* by Muller Hinton Agar Well Diffusion method. The media was prepared under aseptic condition and poured onto the sterile petriplate and allowed to get solidify and the wells were punched using sterile cork borer. The wells were loaded with synthesized colloidal gold nanoparticle solution and plant extract.

H. In Vitro Drug Release Assessment

The synthesized gold nanoparticles were assessed for in vitro drug release by sample and separation method. The 20 µL of human insulin drug was incubated with 50 µL of synthesized colloidal gold nanoparticle solution under continuous stirring by centrifugation for 72 hours. The drug loading into the nanoparticles was monitored by taking the sample 6 hrs intervals and analyzed using UV- Visible spectrophotometer [10, 11]. And the drug loading efficiency was calculated from the formula given below:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of drug in Supernatant}}{\text{Total amount of the drug}} \times 100$$

The drug loaded- gold nanoparticles were incubated with PBS buffer (pH 7.4) in micro centrifuge tube and stirred at 10000 rpm. The samples were collected at selected time interval and replaced with fresh buffer. The drug content was analyzed by using UV-Visible spectrophotometer. A similar work was reported by Ghosh et al. [9].

III. RESULT AND DISCUSSION

Biosynthesis of gold nanoparticles is simple, low cost and eco-friendly method. In the present study, the gold nanoparticles were synthesized using aqueous extract of *Pimpinella anisum* and the presence of bioactive compounds in the plant extract were analyzed (Figure 1). Biomolecules present in the plant extract is given in the table (1) as follows:

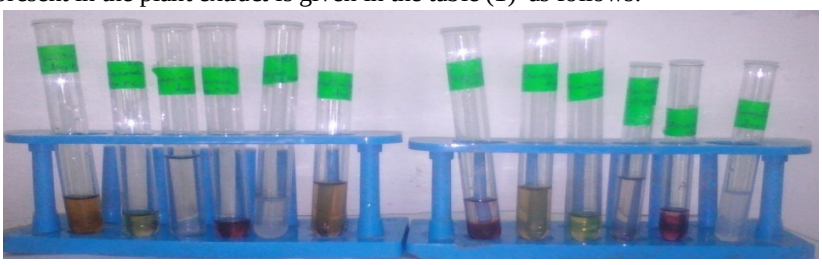

Table 1: Presence Of Bioactive Compounds in Aqueous Extract of *Pimpinella anisum*

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S. NO	PHYTOCHEMICAL	AQUEOUS EXTRACT OF FRESH LEAVES
		Pimpinella anisum
1	Carbohydrates	+
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Alkaloids	-
6	Quinones	+
7	Glycosides	-
8	Cardiac glycosides	+
9	Terpenoids	+
10	Phenols	-
11	Coumarins	+
12	Proteins	+
13	Steroids	+
14	Phylobatannins	-
15	Anthraquinones	-

A. Biosynthesis of Gold Nanoparticles

The gold nanoparticles were extracellularly synthesized by using aqueous extract of *Pimpinella anisum*. The color of the solution mixture changed into ruby red indicates the formation of gold nanoparticles was visually observed. The presence of bioactive compounds in the extract act to reduces Au^{+} ions to Au^{0}. It represents that the bio molecules in the extract are acting as reducing and capping agents.

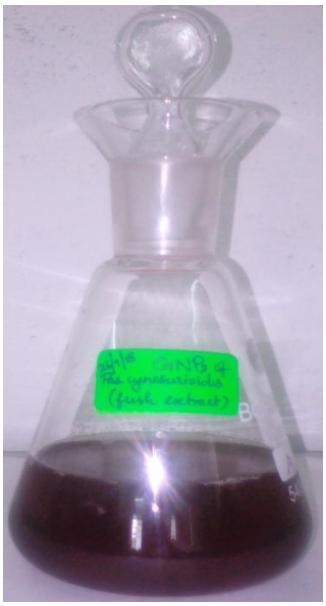

Figure 2 Biosynthesized Gold Nanoparticles using *Pimpinella anisum*

B. Characterization

1) *UV- Visible Spectrophotometer Analysis:* The biosynthesized colloidal gold nanoparticles solution was analysed using UV-Visible spectrophotometer. The SPR band was formed at 534.9 nm.

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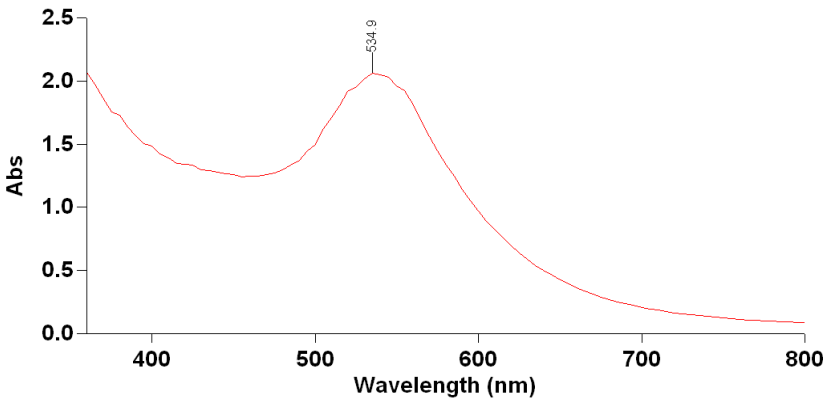

Figure 3 UV- Visible Spectra of *Pimpinella anisum* biosynthesized gold nanoparticles

2) *Scanning Electron Microscope Analysis:* The powder form of biosynthesized gold nanoparticles was analysed using scanning electron microscope. It was found that the gold nanoparticles were spherical in shape and 80-90 nm in size.

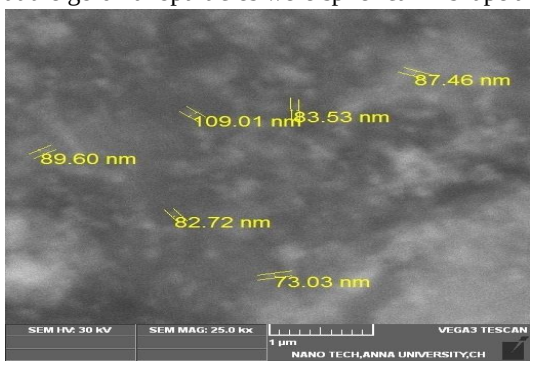

Figure 4 SEM images of Pimpinella anisum biosynthesized gold nanoparticles

3) *X- Ray Diffraction Spectroscopy Analysis:* The powder form of gold nanoparticles were analysed using XRD. The strong peaks were observed at 2θ values of 37.88°, 46.24° and 64.52° correspond to (111), (200) and (220) set of planes and it found to be FCC crystal structure. Thus, the XRD spectrum show that the biosynthesised gold nanoparticles were crystalline in nature. And also the particle size were determined using Scherrer formula and found to be 87.76 nm, 90.26nm and 98.17 nm in size.

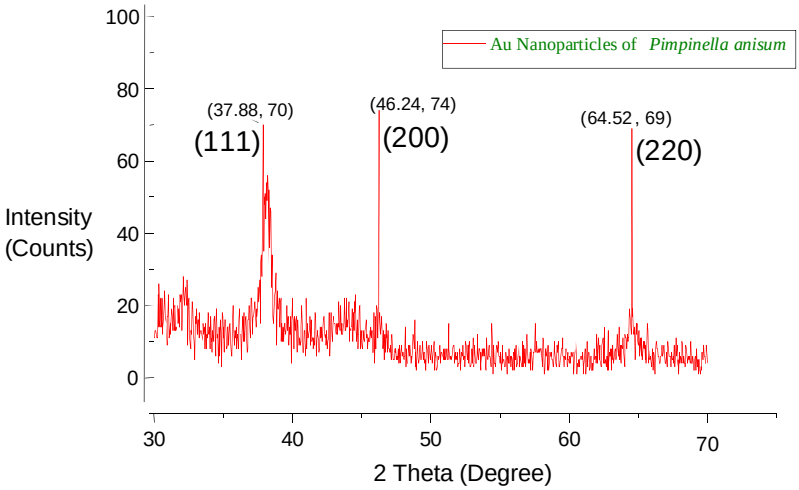

Figure 5 XRD spectra of *Pimpinella anisum* biosynthesized gold nanoparticles

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4) *Fourier Transform – Infra Red Spectroscopy Analysis:* The powder form of gold nanoparticles was made into pellets by ground with KBr disc. The pellets were analysed using FT- IR spectroscopy. The functional groups associated with biosynthesised gold nanoparticles were found (Figure 7), the peak at 3251.98 cm^{-1} indicates the Phenol group (-OH) stretch, the peak at 2922.16 cm^{-1} indicates Alkyl C-H stretch, the peak at 2854.65 cm^{-1} indicates Carboxylic acid –OH stretch, the peak at 1631.73 cm^{-1} indicates Alkanyl C=C stretch, peak at 1546.91 cm^{-1} indicates Aromatic C=C bending, peak at 1375.25 cm^{-1} indicates Nitro compound $–NO_2$, peak at 1236.37 cm^{-1} indicates ASlcohol group and the peak at 1020.34 cm^{-1} indicates Ether (C-O).

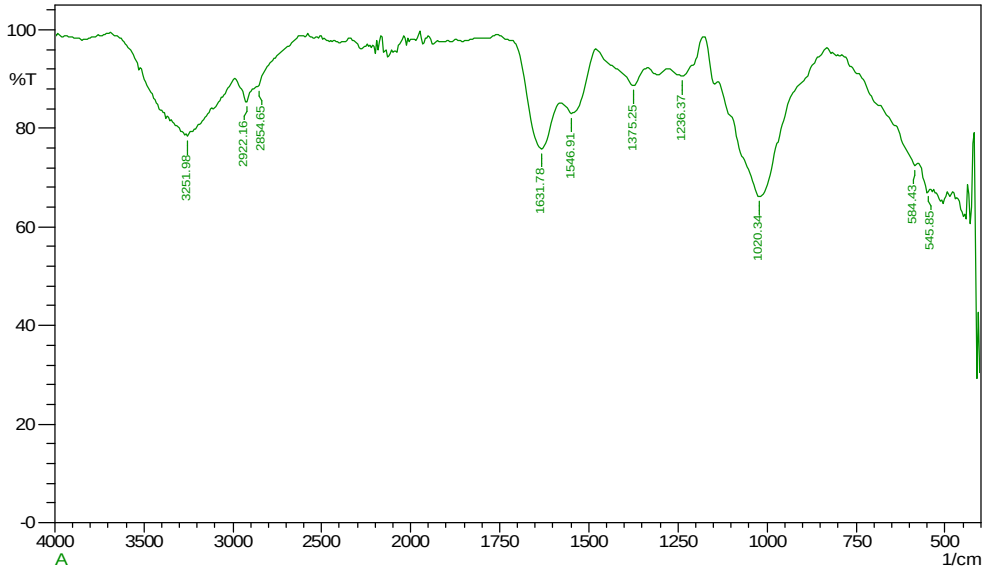

Figure 6 FT- IR spectra of *Pimpinella anisum* biosynthesized gold nanoparticles

C. *Antioxidant Study*

Hydrogen peroxide scavenging assay was used to study the antioxidant property of *Pimpinella anisum* biosynthesized gold nanoparticles. The antioxidant potential of the *Pimpinella anisum* biosynthesized gold nanoparticles registered increase in scavenging activity in according to the concentration increase. The result obtained with *Pimpinella anisum* biosynthesized gold nanoparticles was compared with plant extract and standard ascorbic acid. (Figure 7)

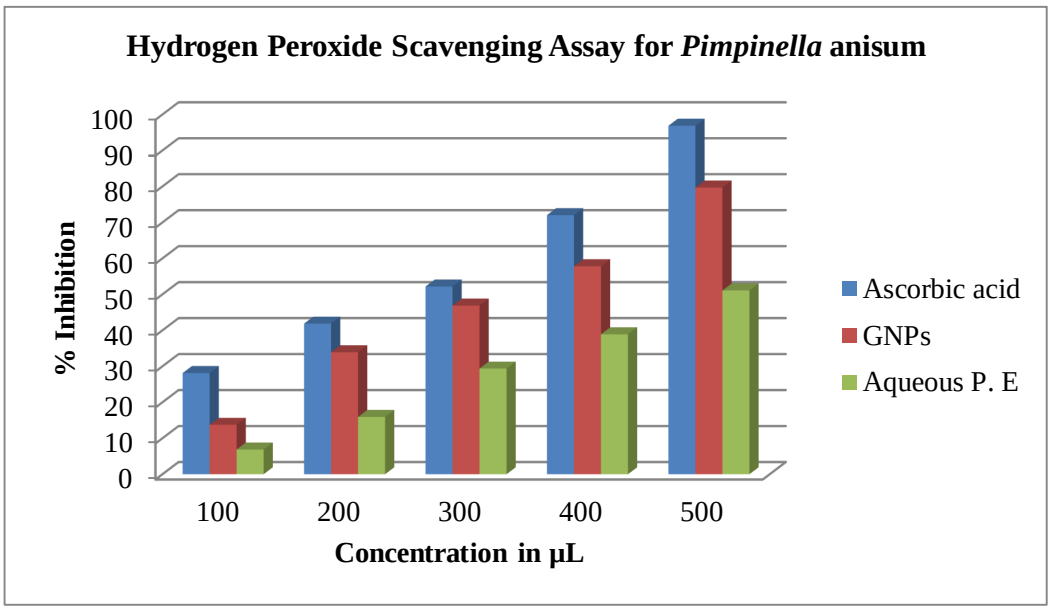

Figure 7 Hydrogen Peroxide Scavenging Assay for *Pimpinella anisum* Biosynthesized Gold Nanoparticles

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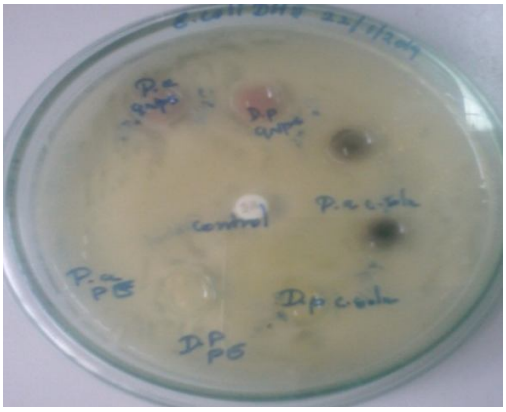
D. Antibacterial Study

Agar well diffusion method was used to study the antibacterial property of the synthesized gold nanoparticles against *E. coli* and *B. substilus* using Nalidiaxic acid as control at centre. The synthesised gold nanoparticles showed high resistance against the growth of microbes and zone of inhibition was measured in mm diameter (Table2).



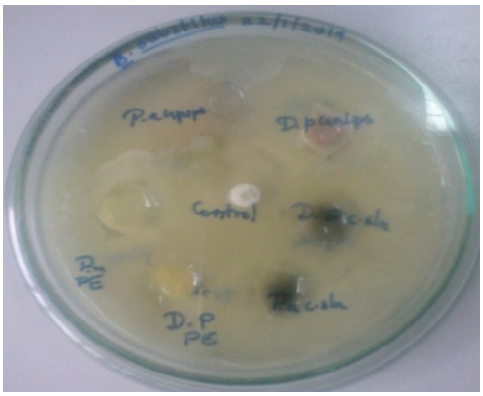


Figure 8 Antibacterial activities of Pimpinella anisum synthesized gold nanoparticles against E. coli Figure 9 Antibacterial activities of Pimpinella anisum synthesized gold nanoparticles against B. substilus

Table 2 : Antibacterial Activity Of Synthesised Gold Nanoparticles Using *Pimpinella anisum* – Zone Of Inhibition In mm Diameter

S. No	Microorganism	Zone Of Inhibition Measurement In mm Diameter	
		Aqueous extract of *Pimpinella anisum*	*Pimpinella anisum* mediated synthesized gold nanoparticles
1.	*Escherichia coli*	8	12
2.	*Bacillus substilus*	13	14
Control: Nalidixic acid= 20mm			

E. In Vitro Drug Release Assessment

The *Pimpinella anisum* synthesised gold nanoparticles were assessed for in vitro drug release of human insulin drug by using sample and separation method. The 89% of drug molecules were loaded onto the synthesised gold nanoparticles were attained. And the release of drug molecules from the drug loaded – gold nanoparticles were analysed using PBS buffer as releasing media under centrifugation and samples were taken at selected interval of time and analysed with UV- Visible spectrophotometer. The 83% of drug release achieved by this method was calculated from the absorbance value.

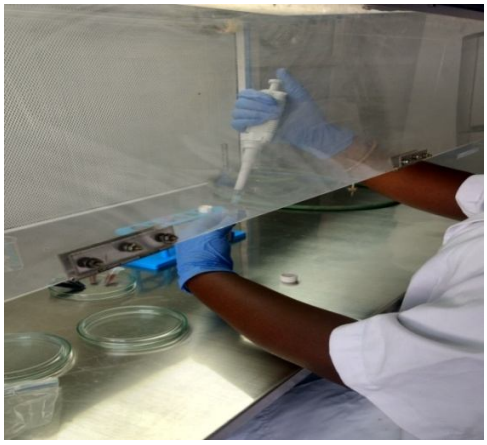


Figure 10 Collection of Supernatant from Drug Loaded Gold Nanoparticles colloidal solution for UV- Visible spectrophotometer analysis

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IV. CONCLUSION

Biological synthesis of nanoparticles has upsurge in the field of nano-biotechnology to create novel materials that are eco-friendly, cost effective, stable nanoparticles with a great importance for wider application in various fields such as electronics, medicine and agriculture. Hence the present investigation deals with extracellular biosynthesis of gold nanoparticles using *Pimpinella anisum* based aqueous extract as reducing and capping agent was demonstrated. The reducing potential of the plant extract is due to the presence of bioactive compounds which act to reduce the gold ions to gold nanoparticles. The color change of the chloro auric acid and extract solution mixture from light green to ruby red was visually observed and confirmed with UV- Visible spectrometer analysis. The SEM image of the synthesised gold nanoparticles showed the spherical shape of the nanoparticles was formed. The XRD analysis was done to know the crystalline nature of the gold nanoparticles and the particle size was calculated using Scherrer formula. It was found to be 87.76nm, 90.26 nm and 98.17nm in size. The FT-IR analysis was performed to know the functional groups associated with the gold nanoparticles. The antioxidant property of the gold nanoparticles is increased with scavenging potential as concentration increases. The antibacterial activity study of the gold nanoparticles showed high sensitivity against the pathogenic microbes. The invitro drug release assessment of the gold nanoparticles was performed using sample and separation method. And the drug loading efficiency of the gold nanoparticles was found to be 89% and the drug release efficiency of the gold nanoparticles was found to be 83%. From the investigation in future, the young nano- biotechnologists can light their research into the biosynthesised gold nanoparticle for drug delivery vehicles.

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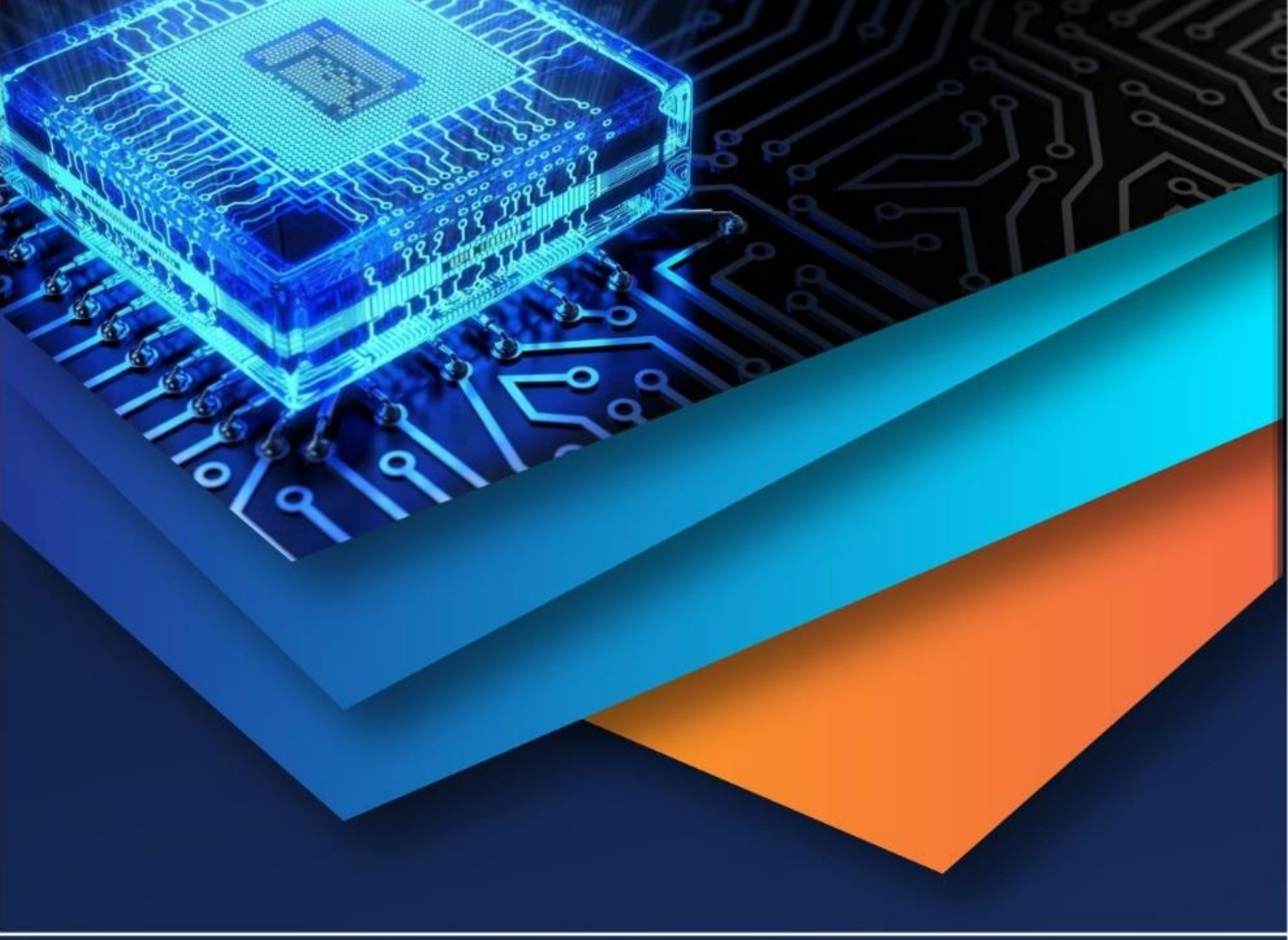

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