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Evaluation of Cytotoxic and Phytotoxic Effect of Silver nanoparticles Synthesized by Banana Peel (Karpooravalli)

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Abstract: ***Natural of silver nanoparticles has now become a novel method to physical and chemical approaches. In the present study, silver nanoparticles (AgNPs) were synthesized from banana peel extract (BPE). Our present research is to investigate on the synthesis eco-friendly silver nanoparticles using banana peel extract. In this natural synthesis we used banana peel extract (Karpooravalli) which act as natural reducing agent to synthesize biodegradable silver nanoparticles. Banana peel extract was found to reduce the silver ions (Ag^+ to Ag_0) and capping agent. The characteristic color of Silver nitrate is changed to reddish brown in the reaction due to reduction by banana peel. The UV-Vis spectrum of silver nanoparticles of our results revealed a characteristic surface plasma- resonance (SPR) peak at 460 nm. X-ray diffraction (XRD) revealed their crystalline nature. Scanning electron microscope showed Monodispersed spherical shaped nanoparticles. The average size of nanoparticles was 10nm as confirmed by Transmission electron microscope. Fourier transform infrared spectroscopy (FT-IR) affirmed the role of BPE as a reducing and capping agent of silver ions. The antibacterial activity of these nanoparticles was also studied against Gram-positive and Gram-negative bacteria. The result of our study suggested that the Cytotoxic and Phytotoxic effect of the green synthesized Silver nanoparticles by banana peel was significantly higher.***

Keywords: Silver nanoparticles, Banana peel extract, Antibacterial, Antifungal, Cytotoxicity, Phytotoxicity.

I. INTRODUCTION

Bio-waste is a form of biomass, capable of decomposing under anaerobic or aerobic conditions. Commercial sources of bio-waste include forestry and agricultural residues, animal waste and manure, sewage sludge and commercial food waste. Household sources of bio-waste include kitchen scraps and garden waste, paper and cardboard, as well as natural textiles. The wastes from fruits and vegetables processing industries being rich in polysaccharides such as cellulose, hemi-cellulose and lignin can be subjected to solid state fermentation for the production of ethanol and butanol, which has several uses (Jorgensen *et al.*, 2007, Lee *et al.*, 2007).Fruits produce two types of waste - a solid waste of peel/skin, seeds, stones etc and liquid waste of juice and wash-waters. In some fruits the discarded portion can be very high eg: mango 30-50%, banana 40%, pineapple 40-50% and orange 30-50%.

For the past decades, efforts have been made extensively to improve methods and find alternative ways to utilize fruits and vegetables wastes therapeutically. Increasing evidences suggest that majority of the bioactive phytochemical components in plants impart physiological activities and may offer a variety of health benefits such as antioxidant, antibacterial, anti-inflammatory and anticancer constituents. Silver nano particles play a pivotal role in the field of biology and medicine. Silver products are known for its sturdy inhibitory and bactericidal effects, in addition to a wide spectrum of antimicrobial activities, which has been practiced to prevent and treat various diseases, most notably infections. The most important and distinct property of nano particles is their larger surface area to volume ratio (Arangasamy Leela and Munusamy Vivekanandan, 2008).An array of physical, chemical and biological methods have been used for synthesis of noble metal nano particles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals (Balagurunathan*et al.*, 2011). The peel of banana represent 40% of the total weight of fresh banana and the peel are often discarded as waste product after the inner flesh portion is eaten. The peel is used for allergies and skin irritations as a good home remedy (Akamineet al., 2009).They are also the good sources of polyphenols, carotenoids, dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium which possess diverse beneficial effects on human health. (Larrauriet al., 1999; Wolfe et al., 2003).Banana peel can be utilized for various useful applications such as bio-fuel manufacturing, bio-sorbents, pulp and paper making, cosmetics, energy related activities, organic fertilizer, environmental cleaning, biotechnology related processes and in nanotechnology (Morton, 1987; Gunaseelan,2004;Boriet al.,2007).Therefore evaluation of anti cancerous and toxicological effect of banana peel cultivar (Karpooravalli) and their relevance in silver nanoparticles synthesis was carried out.

II. MATERIALS AND METHODS

A. *Materials*

Ripened fruits of banana cultivar Karpooravalli was purchased from Kovilpatti market and the peels were carefully removed and used for the analysis.

Vignaradiata (Linn) and Pennisetumtyphoides (L.) R.Br seeds were procured from Agricultural department; Killikulam.

B. *Chemicals*

All the chemicals used in the study were of analytical grade.

Karpooravalli

C. *Methods:*

Preparation of banana peel extract (BPE) Fresh peel of each cultivar was ground with distilled water 1:10 w/v and filtered through cheese cloth and the filtrate was used for the analysis.

D. *Analysis*

In the banana peel extract 1mM $AgNo_3$ is added the reduction of silver nitrate acquired with in 10 mints, which resulted in the colour change (dark brown).As noted by visual observation indicating the formation of AgNPs. As per the observation spectrum, this media remained stable for more than 3 months. This absorption aliquot of the reaction solution was measured using UV-Spectrophotometer (2371) operated at resolution of 1mM.

Different techniques were used to characterize KarpooravalliAgNPs such as UV-visible (2371) used to knew the band at nm , X-ray diffractogram (Modle : XRD analysis (JEOL),with Cu kα 1.5418 A, used to determine the crystallinity success of silver nanoparticles, FT-IR (Fourier Transform Infrared Spectroscopy)(Model: (systronics 166) which is used in the range of 400-40000cm^{-1} by KBr–pellet method. Karpooravalliextract powder synthesized silver nanoparticles. (TEM) (Joel, JEM 2100) ,(SEM)(JEOC JSM 35 CF SEM) analysis were carried out to conform the images of specimen by magnified focus on imaging device. Cytotoxicity of water extracts from Musa paradicicavar. (karpooravalli)were assessed in MCF-7 cells as well as HUVEC using the MTT reagent (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Roche Diagnostics GmbH, Germany) according to the manufacturer's protoco. This method is based on the ability of viable cells to metabolize yellow tetrazolium salt MTT to purple formazan crystals by mitochondrial dehydrogenases. Phytotoxicity-Different seeds of Pearl millet and Green gram were sterilized with 0.01% merchuric chloride to prevent fungal infection after which they were rinsed for about 2 minutes in running water. The seeds were then washed in distilled water, placed on oven dried petridishes lined with two layers of filter papers and moistened with 10 ml of the respective aqueous BPE as test and distilled water as control. The treatments were replicated 3 times. The effect of various percentage of BPE on germination was recorded.

III. RESULTS

Bananas are consumed by all over the world, after consumption of the pulp, the peels are generally discarded. There are reports on the exploitation of banana peel in ethanol fermentation, application as a substrate for generating fungal biomass, used in the production of laccase and utilization as a bio-sorbent for heavy metal removal (Parmar and kan 2008, Bankar*et al*., 2010). In addition banana peels are inherently rich in polymers such as lignin, cellulose, hemicelluloses and pectin and minerals especially potassium. However there are no reports on antioxidant potentials of commonly cultivated cultivars of Thoothukudi district and their utilization in silver nano particles synthesis are also not thoroughly investigated. As a step towards novel utilization of bio-wastes, the present study has been under taken, utilizing the peel of different cultivars of banana.

Study on the utilization of banana peel in biosynthesis of silver nanoparticles

Silver is a nontoxic and safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms (Jeong et al., 2005). There is an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display (Choi et al., 2008). They are even being projected as future generation antimicrobial agent (Rai et al., 2009). Synthesis of nanoparticles by physical and chemical methods may have considerable environmental destruction, technically laborious and economically expensive (Gopinath et al., 2012). The biological methods, using microorganisms and enzymes, have been suggested as possible eco-friendly alternatives (Mohanpuria et al., 2008). The plants or plants extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes, because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis (Saxena et al., 2012,). Different parts of plant materials such as leaf extracts (Mubarak Ali et al., 2011), fruit, bark (Satishkumar et al., 2009), fruit peels (Bankaret al., 2010), root (Ahmad et al., 2010) and callus (Nabikhanet al., 2010) have been studied for the synthesis of silver, gold, platinum and titanium nanoparticles in different sizes and shapes.

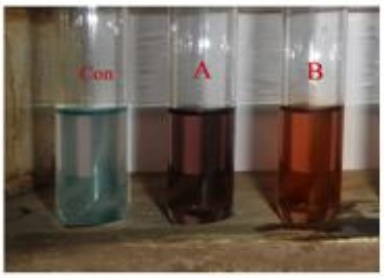
It is generally recognized that silver nanoparticles attached to the cell wall of micro organisms thus disturbing cell wall permeability and cellular metabolism. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorous and sulphur containing biomolecules such as DNA and protein. Hence exploitation of biowaste , banana peel for the biosynthesis of nanoparticles develops a new insight in the field of Pharmacology.

A. Visual observation and uv-visible spectroscopy study

The colour of the reaction mixture started changing to yellowish brown with in 15mints and reddish brown after 24hrs,indicating the generation of silver nanoparticles ,due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag^{o} via the active molecules present in the banana peel extracts (BPEs) (plate 2). This colour formation is attributed to the excitation of Surface Plasmon resonance (SPR) exhibited by the metal ion (Ahmad *et al.,* 2003).As shown in Fig(1) a characteristic and well defined SPR band for silver nanoparticles synthesized by using karpooravalli extract as reducing agent was obtained at around wave length 410-440nm.(fig1-5)**,** confirming the formation of silver nanoparticle. (Baia and simon, 2007, Aymane*t al*., 2014).

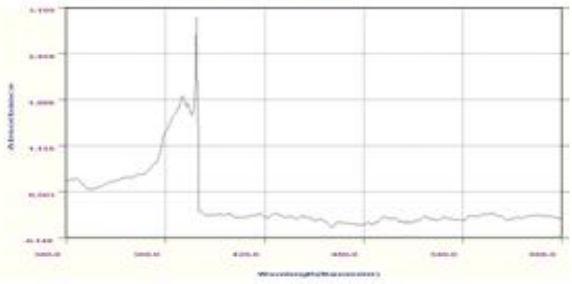
Plate 2

Digital photograph showing the colour change from pale yellow to brown after 24hrs by addition of silver nitrate (1mM) with aqueous peel extracts of Karpooravalli



(A) after 24 hrs (B) after 48 hrs of incubation

Fig.1 UV-Vis spectrum of AgNO3 synthesized using fresh peel extract of Karpooravalli



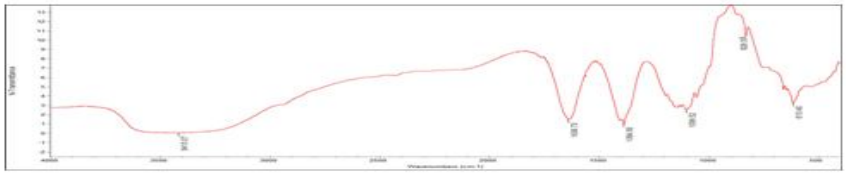
At room temperature (30°C), the colour change took 24 hours to develop, while by heating the reaction mixture to 90°C the reduction process was faster and the reddish brown colour was formed within 10 minutes, (Plate 2) is in corroboration with earlier reports (Haytham and Ibrahim 2015). Similarly, it was documented that the size of silver nanoparticles was decreased with an increase in the incubation temperature (Fayazet al., 2009). By this study utilization of fruit peel for the consistent and quick synthesis of silver nanoparticles was successfully demonstrated. Silver nanoparticles synthesized with banana peel extract was assumed to be smaller and larger size based on UV-visible spectroscopic spectrum. Of the varieties of banana, a peel of karpooravalli was found to be more effective in mediating the production of smaller size silver nanoparticles. The study revealed

that utilization of banana peel is a promising alternative for green synthesis of silver nanoparticles. Therefore BPE mediated silver nanoparticles were further subjected to FTIR, SEM, and TEM analysis and characterized.

B. Ftir

Fourier Transform Infrared Spectroscopy is an analytical technique used to identify the functional groups of the organic materials .This technique measures the absorption of infrared light at various wavelengths by the materials of interest. (Silverstein and Webster ,1999).Several indicator bands in FTIR spectrum that are pertained to functional groups represent the chemical components or metabolic products. FTIR analysis was conducted on the biosynthesized silver nanoparticles using banana peel extracts (BPEs) of karpooravalli and the IR spectra are shown in Fig (2) . A sharp peak at 3415.07 cm^{-1} can be attributed in the peel extracts of karpooravalli AgNPs was assumed to be due to the N-H stretching vibrations .1636.73 cm^{-1} to –C=C stretching vibrations of alkanes. (Chatjigakis 1998;Kadha,2005).A weak peaks are at 1384.56 cm^{-1},1099.52 cm^{-1},828.33 cm^{-1}, 613.40 cm^{-1} accounted to –C-C- ,stretching vibrations of aliphatic amines or to alcohols and phenols indicating that phenols also play an important role in the reduction of silver nitrate. The finger print thus obtained suggests that bound and unbound amide linkages belonging to aromatic ring, ethers and polyphenols are bioactive components that act as reducing and stabilizing agents towards the biosynthesized AgNPs. (Prathna, T.C., *et al.*, 2011). The bioactive components such as antioxidants, flavonoids, terpenes acids, sugars and proteins may also be involved in reducing silver nitrate as well as capping the biosynthesized silver nano particles.(Krishnaraj .C.,*et al.*, 2010).

Fig :2 FT-IR Spectrum of silver nanoparticles synthesized by using aqueous banana peel extract (Karpooravalli).

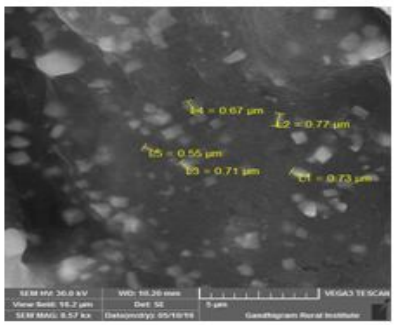


IR absorption / cm	Intensity	Hydrocarbon groups
613.40	Weak	-C-C alkane
828.33	Weak	-C-C alkane
1099.52	Weak	-C-C alkane
1384.56	Weak	-C-C alkane
1636.73	Variable	-C=C-alkene
3415.07	Medium	N-H-Amine

C. Sem

The SEM image of the AgNPs is shown in Plate (3). It was seen that AgNPs formed were of spherical shaped in case of cultivar of banana peel extract (BPE) karpooravalli being used as reducing and capping agents .This may be due to availability different quantity and nature of capping agents present in the different cultivars of BPEs. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis.

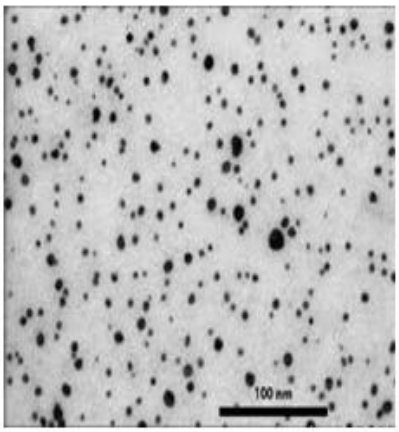
Plate: 3 Scanning Electron Microscopy (SEM) micrographs of silver nano particles synthesised by using aqueous peel extract of banana cultivar-Karpooravalli



D. Tem

The image shows the presence of spherical shaped nanoparticles. However the size of the nanoparticles differes in banana peel extracts (BPEs).The peel extracts of peel extracts of karpooravalli mediated the formation of spherical shaped nanoparticles of sizes upto 100nm.(Plate 4).

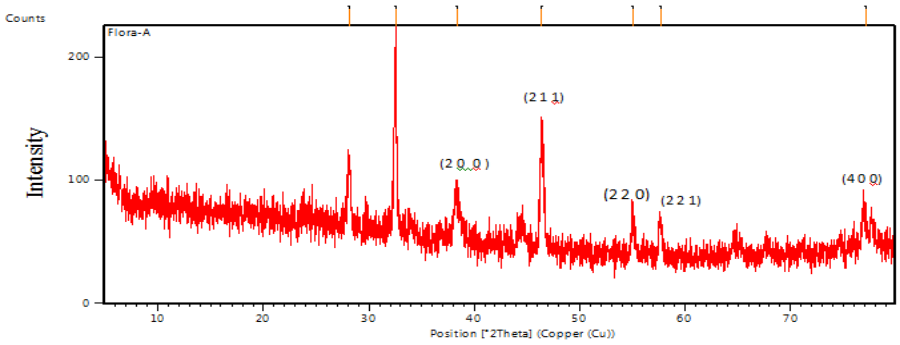
Plate: 4 TEM micrographs of the silver nanoparticles synthesized by using aqueous banana peel extract



E. X-Ray Diffraction

The X-RAY diffraction (XRD) pattern clearly showed that BPE mediated Ag nanoparticles were crystalline in nature. The X-RAY diffractogram of karpooravalli mediated AgNPs exhibited seven intense peaks in the whole spectrum of 2θº values of 28º,32º,38º,46º,55º,57º,77º. A number of Bragg's reflections corresponding to (1 1 1) (2 0 0) (2 1 1) (2 2 0) (2 2 1) and (4 0 0) sets of lattice planes were noted . A crystal is composed of periodically arranged atoms in 3D space. On the other hand amorphous materials do not possess that periodicity and the atoms are randomly distributed in 3D space. When there is periodic arrangements of atoms the X-ray will be scattered only in certain directions, thus conforming the crystalline nature of BPE-AgNPs.

Fig:3 X-ray diffractogram of silver nanoparticles synthesized by using peel extracts of Karpooravalli.



F. Cytotoxicity (Anticancerous) study

Cancer remains one of the world's most devastating diseases and current cancer treatments include surgical intervention, chemotherapeutic drugs and radiation, which often also kill healthy cells and cause toxicity to the patient (Pulit et al., 2011), the new therapeutic methods should have fewer side effects for cancer patients. Silver nanoparticles are widely investigated for biological applications and medical purposes due to their unique optical properties and electrochemical stability (Gericke.M, et al., 2006).Therefore, in the present study, silver nanoparticles were synthesized using karpooravalli banana peel extracts and the apoptotic effects of capped AgNPs against cervical carcinoma HeLa cells were studied.

MTT assay was performed to find out the carcinoma cells (MCF-7) viability and death in response to silver nanoparticles treatment. As shown in plate (4) reduction in cell viability by ~50% (inhibitory concentration=IC50) in comparison with the control was achieved at a dose of100 µg/ml of AgNPs, while the greatest number of cells were killed at 200 µg/ml of AgNPs. It is reported that treatment with Hela cells with AgNPs for five days lead to fragmentation of DNA and the cells underwent apoptosis owing to the effective toxicity exhibited by BPE mediated AgNP. The banana peel could be explored in future as a raw material for nanoparticle synthesis .It is ensured that this work will create a new horizon in the utilization of bio-waste.

Table 1: Anticancerous activity of banana peel extract

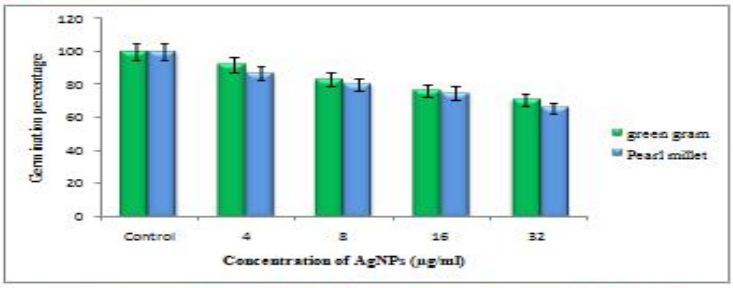
S.NO.	Concentrations (µg/ml)	Cell viability (%)	Cell death (%)
1	Control	100 ± 0	0 ± 0
2	50	75.32±0.38	24.68±0.42
3	100	52.32± 0.30	47.68±0.39
4	150	23.42 ± 0.65	76.58±0.24
5	200	6.33 ± 0.21	93.67±0.73

Values are mean of 3 replicate ± SD.
Peel extract of Karpooravalli (cultivars) was used for the study. Water was used as extractant.

G. Phytotoxicity Study

Nano technological research has increased strongly and attracted many scientists towards it, but its consequences are poorly known. Recently negative impacts of different nanoparticles on the environment have been studied and its toxic potentials in the environment have been ascertained. Therefore , toxicity analysis of the AgNPs prepared by using BPE was carried out on green gram and pearl millet . Seed germination and their resultant root and shoot lengths were recorded. Seeds were considered to have germinated by observing the emergence of radicles .Maximum germination of seeds was obtained after 24hrs.Results obtained varied significantly with each treatment.100% germination was observed in chosen species under normal condition (water treatment). Seeds of green gram were soaked and treated with low concentration (4µg/ml) of silver nanoparticles showed 92% germination where as at higher concentration the germination percentage was reduced (76% at 32µg/ml concentration). Similar trend was noticed in pearl millet however the extent of toxicity was higher than green gram. The response to AgNPs also differed with test seeds suggesting the genotype tolerance variability. In correspondence to germination, seedling establishment was also affected by BPE mediated AgNP. Shoot growth was reduced in comparison with control and was concentration dependent in both species. Similarly, root growth was also decreased with increasing concentration of BPE mediated AgNP. The toxicity was more pronounced in pearl millet than green gram. Adverse effect of silver nanoparticles in edible crop plants is also reported in Phaseolusradiatus and Sorghum bicolor. It was reported in agar test that Phaseolusradiatus and Sorghum bicolor showed a concentration dependent growth inhibitions by silver nanoparticles.(W.M Lee et al., 2012). Phytotoxicity of silver nanoparticles was also reported in Oryza sativa. The present work brought out that silver nanoparticle.

Fig:4 Effect of varying concentration of silvernano particles on germination of green gram and pearl millet.



Germination was recorded on 2nd day and 3rd day for Green gramand Pearl millet respectively.AgNP was synthesized by using Karpooravalli peel extract.

Table: 2 Effect of varying concentration of silvernano particles on shoot length of green gram and pearl millet.

Concentration of AgNPs(µgm)	Shoot length (cm)					
	Green gram			Pearl millet		
	2th day	5th day	10th day	3rd day	7th day	10th day
Control	2.9	4	5.9	3.7	4.5	5.2
4	2.5	2.8	5.3	2.7	3.5	3.9
8	2.7	3.7	4.4	2.4	2.7	2.8
16	1.3	1.7	3.2	1.9	2.3	2.4
32	1.5	1.8	2.1	0.8	1.1	1.5

Shoot length was recorded for Green gram and Pearl millet AgNP was synthesized by using Karpooravalli peel extract.

Table: 3 Effect of varying concentration of silvernano particles on root length of green gram and pearl millet.

Concentration of AgNPs(µgm)	Root length (cm)					
	Green gram			Pearl millet		
	2th day	5th day	10th day	3rd day	7th day	10th day
Control	1.6	2.4	3.7	2.1	3.5	3.8
4	1.4	1.8	3.5	1.7	2.2	2.5
8	1.2	1.4	2.4	1.3	2.5	3.1
16	0.7	1.1	1.4	0.9	1.3	2.0
32	0.5	0.9	0.9	0.7	1.5	1.8

Root length was recorded for Green gram and Pearl millet AgNP was synthesized by using Karpooravalli peel extract.

IV. CONCLUSION

The study brought to light that bio-wasteie., banana peel of different local cultivars is a viable resource for antioxidant extraction, isolation of antimicrobials, anticancerous drugs production of smaller size, spherical shaped nanoparticles. Although significant value of AgNP were revealed ,further research is required to safely exploit the tremendous potentials of green nanoparticles without jeopardizing human health, crop growth and production, and the environment.

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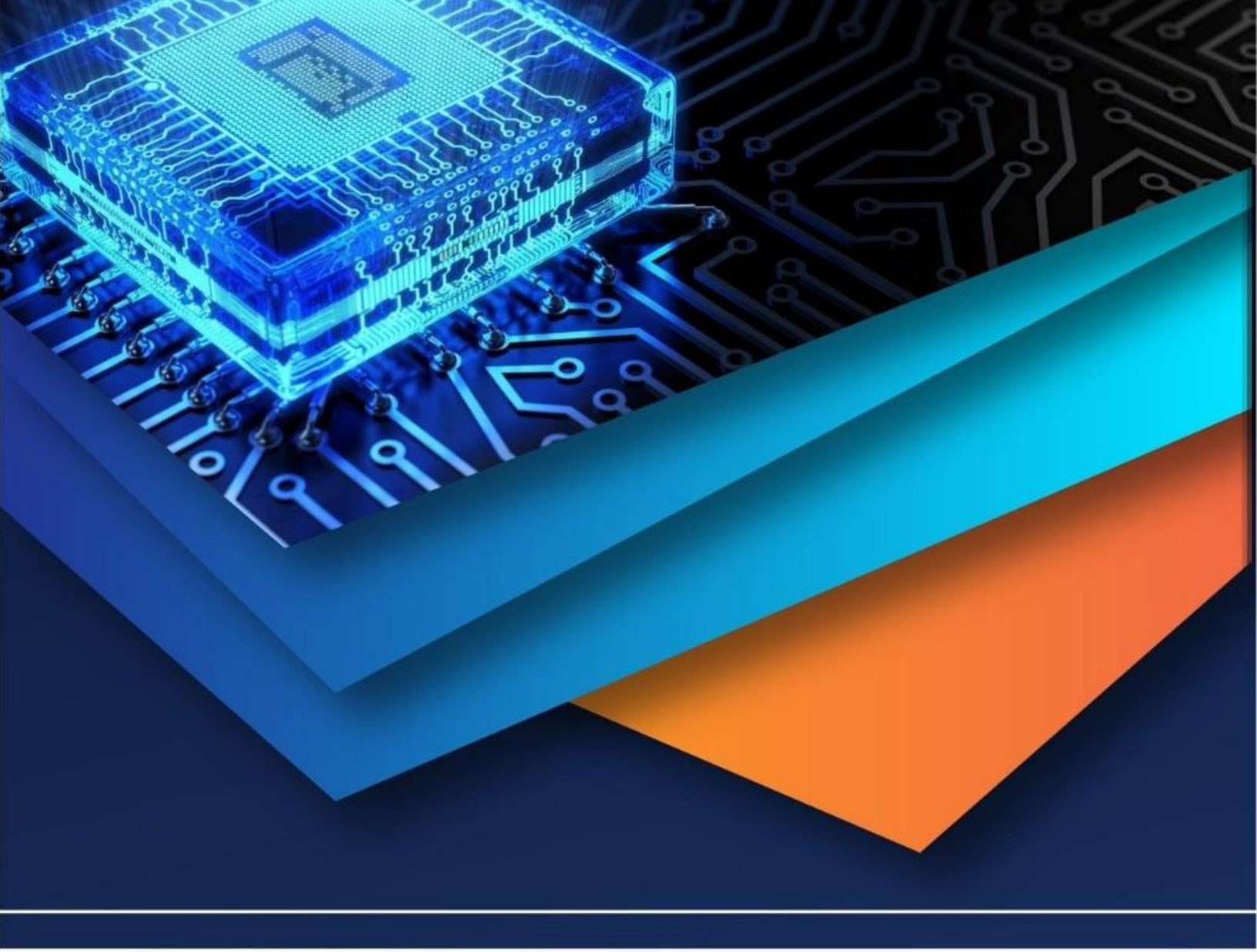

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