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Effect of Silver Nanoparticles Synthesized from Daemia Extensa against the Dengue and Chikungunya Vector, Aedes Aegypti

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Abstract: ***Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and other animals. In addition to nuisance, vector-borne diseases cause thousands of deaths per year. India reports millions of malaria, chikungunya , Japanese encephalitis cases and dengue cases. The World Health Organization estimates that there may be 50–100 millions of dengue infections worldwide every year. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people over 40% of the world's population are now at risk from dengue. Application of plants for synthesis of nanoparticles can be advantageous over other biological processes. Green nanoparticle synthesis has been accomplished using bicompible plant extract, reducing and capping agents. The larvicidal activity of biosynthesized silver nanoparticles using the plant extracts has been tested against the dengue and chikungunya vector, Aedes aegypti. The synthesised silver nanoparticles were characterized by UV-Visible spectroscopic analysis and the peak is observed at 437 nm and it is achieved within 24 hrs, The SEM analysis proved that the shape of the nanoparticles is spherical, FTIR analysis showed the functional group of synthesized silver nanoparticles belongs to free amino or carboxyl group and LC_{50} value of aqueous leaf extract is 51.731 ppm and the LC_{50} value of silver nanoparticles synthesized from the leaf extract of Daemia extensa is 3.842 ppm respectively.***
Keywords: Aedes aegypti, Daemia extensa, larvicidal, pupicidal, dengue, chikunqunya.

I. INTRODUCTION

Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and other animals. In addition to nuisance, vector-borne diseases cause thousands of deaths per year. India reports millions of malaria, chikungunya, Japanese encephalitis cases and dengue cases [1]. Dengue fever incidence has increased fourfold since 1970, and 1.5 billion people, nearly half the world's population, lived in regions where the estimated risk of dengue transmission was greater than 50 % [2]. Aedes mosquitoes on the other hand are also painful and persistent biters. Ae. aegypti is responsible for spreading dengue. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people over 40% of the world's population are now at risk from dengue. Many medicinally important plant extracts have been studied for their efficacy as mosquito agent against different species of vector mosquitoes. Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and do not induce pesticide resistance in mosquitoes [3]. In addition to the direct use of phytoextracts, in recent days, biosynthesized silver nanoparticles gained momentum as bio control agents against mosquitoes and microbes. In these circumstances, improvised methods using the biologically synthesized silver nanoparticles were evaluated for the destruction of the mosquito larvae of Aedes albopictus. Silver nanoparticles are emerging as one of the fastest growing material due to their unique physical, chemical and biological properties, small size and high specific surface area [4]. Nanoparticles are very vital in all fields of modern sciences including biology, chemistry, physics, electronics, biotechnology, and medicine. A nanoparticle shows properties which are built on certain features such as shape, size, scattering, and morphology [5]. Application of plants for synthesis of nanoparticles can be advantageous over other biological processes because it get rid of the complex process of maintaining cell cultures and is also suitable for large scale nanoparticle synthesis. Hence, the present investigation was aimed to synthesis of silver nanoparticles using aqueous leaf extract of Daemia extensa and evaluate of its larvicidal activity against dengue and chikungunya vector.

II. MATERIALS AND METHODS

The fourth instar larvae of the Aedes aegypti mosquito was treated with the aqueous leaf extract and the green nanoparticles synthesized from the leaves of Daemia extensa.

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A. Preparation of Plant Extract

The freshly harvested plant leaves were washed thoroughly in tap water, pat dried with paper towel, and shade-dried at room temperature (35 ± 1^{o} C). These dried leaves were powdered mechanically using electrical mixer. Aqueous extract was prepared by mixing 10g of dried leaf powder in 100 ml of double distilled water. This suspension was mixed well and left for 5 hours without disturbance, then filtered through Whatman No. 1 filter paper. The filtrate was used to find out the larvicidal and pupicidal activity against the target vector.

B. Silver Nitrate Preparation

Silver nitrate was used as precursor for the synthesis of silver nanoparticles. Analytical grade, silver nitrate ($AgNO_3$) was prepared for 16.96 mg of silver nitrate was carefully weighed and dissolved in 90 ml of Milli-Q-water. This aqueous Silver nitrate solution was always prepared fresh.

C. Collection and Maintenance of Target Vector

Different larval instars and pupa of *Ae. aegypti* were collected from the Indian Council for Medical Research, Madurai and were brought to the laboratory safely without disturbance. These larvae and pupae were maintained in enamel trays containing deionized water and allowed to feed on brewer's yeast, dog biscuits and sucrose in a 3:1:1 ratio in the laboratory at room temperature for 24 hours, before start of the experiment.

D. Synthesis of Silver Nanoparticles From Leaf Extract

Aqueous leaf extract of Daemia *extensa* was prepared by placing 10 g of chopped fresh leaves in a 250 ml Erlenmeyer flask and boiled with 100 ml of sterile double distilled water up to 60 min at 60^{0}C in a water bath. The crude extract was passed through Whatmann filter paper (no.1), and the filtrates (=aqueous leaf extract) were stored at 4^{0}C and used within 3 days. Ten millititre of aqueous leaf extract was treated with 90 ml of prepared 1mM aqueous Ag NO_3 Solution in an Erlenmeyer flask and incubated in dark at room temperature. The aqueous solution of 1mM of Ag NO_3 was leading to change of pale yellow to dark brown resulting in synthesis of Ag NPs.

III. CHARTCTERIZATION

A. UV-Visible Spectral Analysis

Initial Characterization of silver nanoparticles was carried out using UV-Visible spectroscopy. The bio-reduction of silver ions to silver was monitored by measuring the UV-Vis spectrum of the reaction mixture (silver nitrate+ aqueous leaf extract). This reaction mixture (1ml) was drawn at different time interval (min), and the absorption measurements were carried out on UV-Visible spectrophotometer at a resolution of 1nm between 200-800 nm.

B. Particle size analyzer

The particle size of the synthesized silver nanoparticles was characterized by laser diffraction Particle Size Analyzer (Shimadzu model and model no: 2300). The particle size was determined using the scattered light intensity pattern.

C. Fourier Transform Infra Red Spectroscopy

A dry nanoparticle powder was obtained in the following manner. Silver nanoparticles synthesized after 5 hours of reaction of mM $AgNO_3$ solution with M.bipinnatifida extract centrifuged at 10,000 rpm for 15 minutes at room temperature, after which the pellet was redispersed in sterile distilled water. The process of centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the nanoparticels. The nanoparticle powder was mixed with KBr and exposed to an infrared source of 500-4000 cm^{-1}.

D. Scanning Electron Microscopy

Scanning Electron Microscopic (SEM) analysis was done using Hitachi-S-4500 SEM machine. Thin films of the sample were prepared on a carbon 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 film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

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E. Larvicidal and Pupicidal Activity

The larvicidal and pupicidal activity was evaluated using WHO method (1996) with slight modifications. Different test concentrations of leaf extract and AgNPs in 200 ml de-ionized water were prepared in 250 ml capacity autoclaved glass bottles. Bio – efficacy test was conducted against the larvae and pupa of target vector at ten different concentrations of aqueous leaf extract and synthesized AgNPs, 10 larvae were exposed to each test at different concentration. Similarly, each test included a set of control group (distilled water) with ten replicates for each individual concentration. Mortality rate was recorded after 24 h of exposure period. The dead larvae in ten replicates were combined expressed as a percentage of larval and pupal mortality for each concentration.

F. Statistical Analysis

The results obtained were subjected to statistical analysis to ascertain their credibility. Standard deviation and mean separation statistical tools were employed for analysis of larval and pupal mortality obtained in the present investigation using computer software. The dose response mortality data were concerned to probit analysis for finding the $LC_{50,}$ upper and lower confidence limit at 95 % confidence, and values determined using the software (Probit analysis, Finny method SPSS 2007).

IV. RESULTS AND DISCUSSION

A. UV- Visible Spectroscopy Analysis

The synthesis of silver nanoparticles using leaf extract was further confirmed by UV-Visible spectroscopy. The UV-Visible spectra showed an absorption band at 437.50 nm (Fig.1). Similar observations were reported in previous studies, for silver nanoparticles it is around 480 nm. [6], 450 nm for silver nanoparticles of Polianthus tuberose [7], 422 to 447 nm for silver nanoparticle of Cardiospermum halicacabum [8].

B. The particle size Analyzer

The particle size of the synthesized silver nanoparticles was characterized by laser diffraction Particle Size Analyzer (Shimadzu model and model no: 2300). The particle size was determined using the scattered light intensity pattern. The light intensity pattern thus clearly shows that the silver nanoparticles are crystalline in nature due to the reduction of Ag+ ion by Daemia extensa leaf extract. The size of the silver nanoparticles synthesized from Daemia extensa was predicted as 0.545 nm (Fig.2). Similar results were reported in Azhadirachta indica [9].

C. SEM Analysis of silver nanoparticles

The morphology and size of the silver nanoparticles was investigated by scanning Electron Microscope (SEM). SEM analysis revealed that the particles were mostly aggregated and spherical in shape (Fig. 3). Similar shapes of nanoparticles were synthesized from Ziziphus nummularia by [10]. They also reported that the synthesized silver nanoparticles were spherical in shape.

D. FTIR Analysis

Fourier transform infrared (FTIR) spectroscopy measurements were obtained dry powders of the nanoparticles. The FTIR spectrum of produced silver nanoparticles had many absorption bands (Fig. 4) and the absorption bands seen at $595cm^{-1}$, $656.72cm^{-1}$, $748.33cm^{-1}$, $1076.21cm^{-1}$, $1121.53cm^{-1}$, $1200.61cm^{-1}$, $1386.72cm^{-1}$, $1417.8cm^{-1}$, $1639.38cm^{-1}$, $2503.43cm^{-1}$, $3053.11cm^{-1}$, $3520.81cm^{-1}$. Similar results were reported by [11] the spectrum exhibits the bands at 1418 cm^{-1} corresponding to aromatic group. Proteins present in the extract can bind to AgNP through either free amino or carboxyl groups in the proteins.

E. Larvicidal and Pupicidal Activity

The effect of leaf extract of Daemia extensa on the survival and development of fourth instar larvae of the Dengue and chikungunya vector, Aedes aegypti using probit analysis. LC_{50} value of aqueous leaf extract is 51.731 ppm and the LC_{50} value of silver nanoparticles synthesized from the leaf extract of Daemia extensa is 3.842 ppm respectively (Table 1). The larval and total mortality increased along with the increasing concentration of aqueous and silver nanoparticles synthesized from leaf extract of Daemia extensa (Table 2 & Table 3). Similar results were observed by [12] in the aqueous plant extract of Daemia extensa against the fourth instar larvae Aedes aegypti. High larval mortality (80-100%) was noticed in mixture treatment, V. negudo, Z. officinalis and O. santum which may be due to the chemical constituents present in leaf and seed extracts that arrest the metabolic activities of larvae.

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Table 1. LC_{50} value of the test solutions for Aqueous leaf extract and AgNPs synthesized from leaf extract of Daemia extensa against IV instar larvae of the mosquito Aedes aegypti.

Test solution	LC_{50} values
Aqueous extract of leaves Daemia extensa	51.731ppm
AgNPs synthesized extract of leaves of Daemia extensa	3.842ppm

Table 2. Effect of different concentrations of aqueous leaf extract of Daemia extensa (L) on the larval and pupal period, larval, pupal and adult mortality % of total mortality and adultemergence on the IV instar larvae of Ae.aegypti.

S.No	Parameter	Control	Concentration of aqueous leaf extract in ppm									
			10	20	30	40	50	60	70	80	90	100
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	1	3	4	5	7	7	8	9	10
4	Pupal mortality	0	1	2	2	3	1	0	0	0	0	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	20	30	50	70	60	70	70	80	90	100
7	Adult Emergence %	100	80	70	50	30	40	30	30	20	10	0

Table 3. Effect of different concentrations of AgNPs synthesized leaf extract of Daemia extensa (L) on the larval and pupal period, larval, pupal and adult mortality % of total mortality and adult emergence on the IV instar larvae of Ae.aegypti.

S.No	Parameter	Control	Concentration of AgNPs synthesized in leaf extract in ppm									
			1	2	3	4	5	6	7	8	9	10
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	2	4	5	5	6	7	8	9	9	10
4	Pupal mortality	0	2	2	1	2	2	0	0	0	0	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	40	60	60	70	80	70	80	90	90	100
7	Adult Emergence %	100	60	40	40	30	20	30	20	10	10	0

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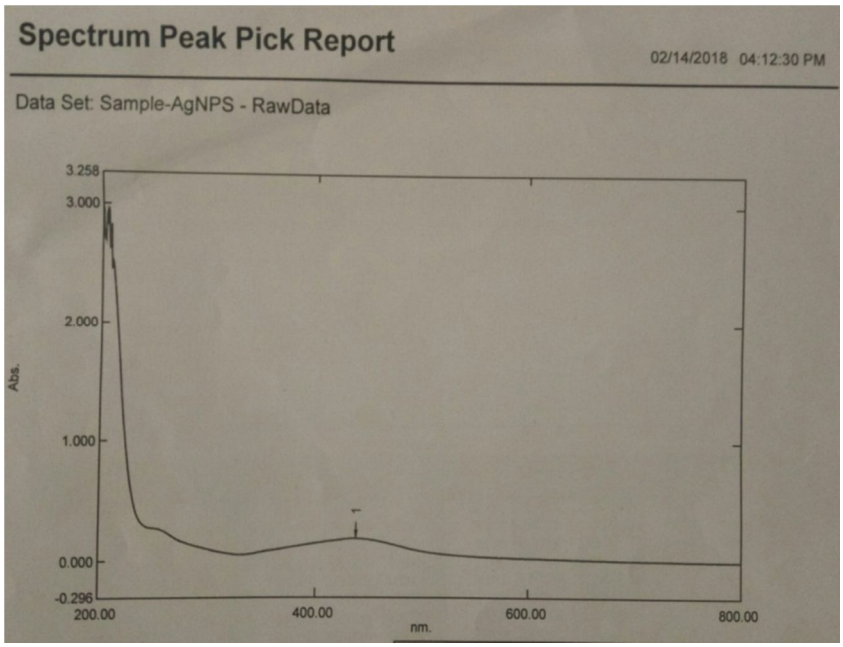


Fig . 1 UV-Visible absorption spectrum of silver nanoparticles synthesized from leaf extract of Daemia extensa.

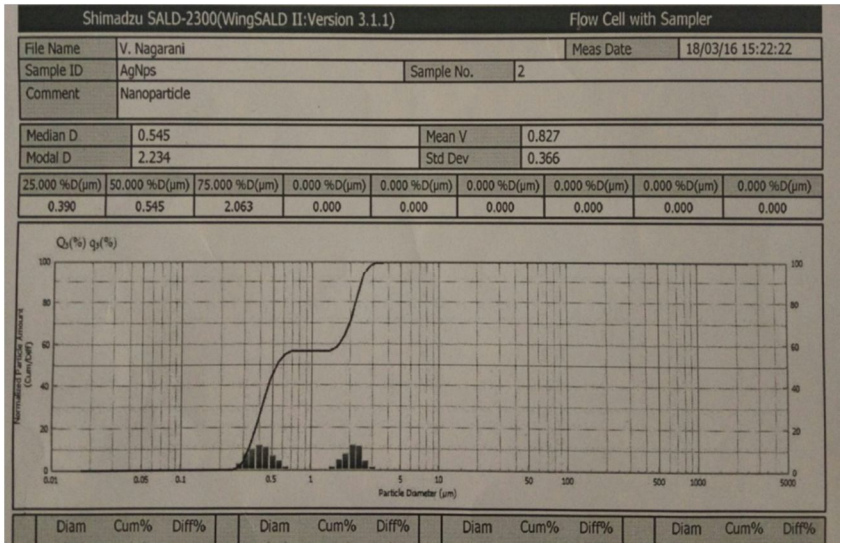
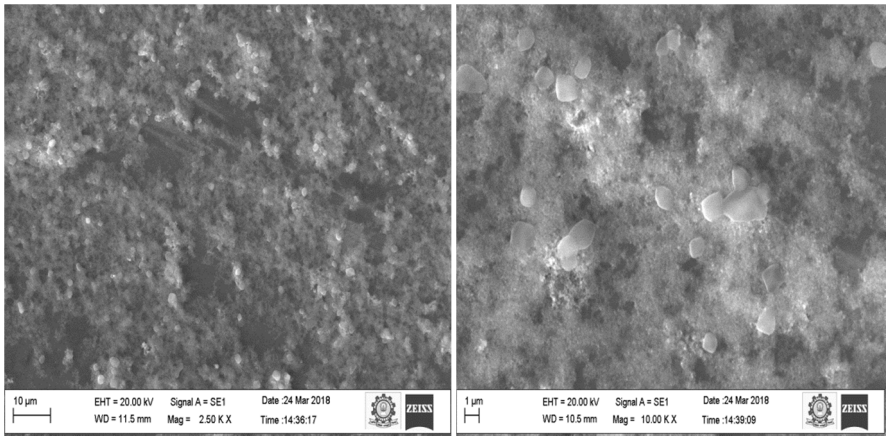


Fig. 2 Particle size analyzer Patterns of synthesized silver nanoparticles using leaf extract of Daemia extensa.



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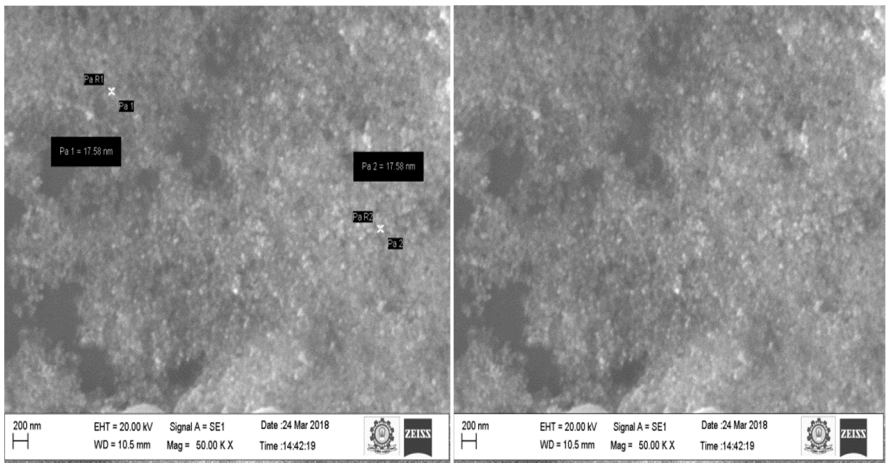


Fig. 3 SEM image of synthesized silver nanoparticles using leaf extract of Daemia extensa.

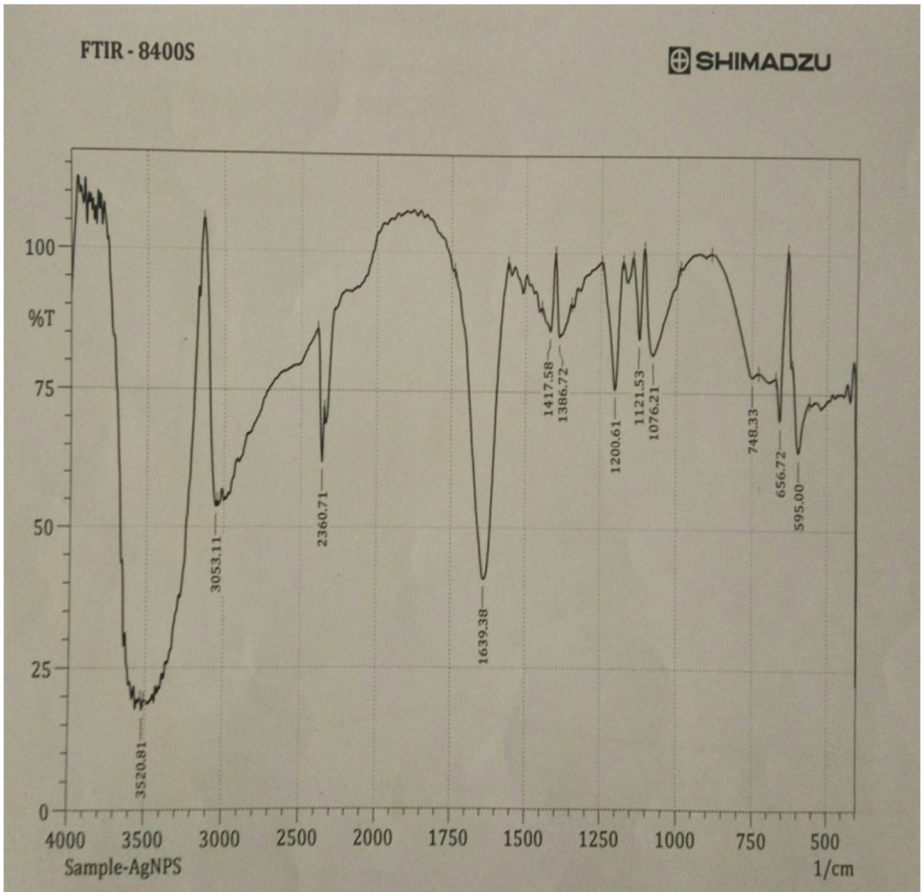


Fig. 4 FTIR spectrum of synthesized silver nanoparticle

IV. CONCLUSION

The synthesized silver nanoparticles from the leaf extract Daemia extensa were more efficient than the aqueous leaf extract. Thus the present study also suggested that the Daemia extensa leaf mediated siver nanoparticles could be used as effective larvicidal and pupicidal agents for the management of mosquitoes.

V. ACKNOWLEDGEMENT

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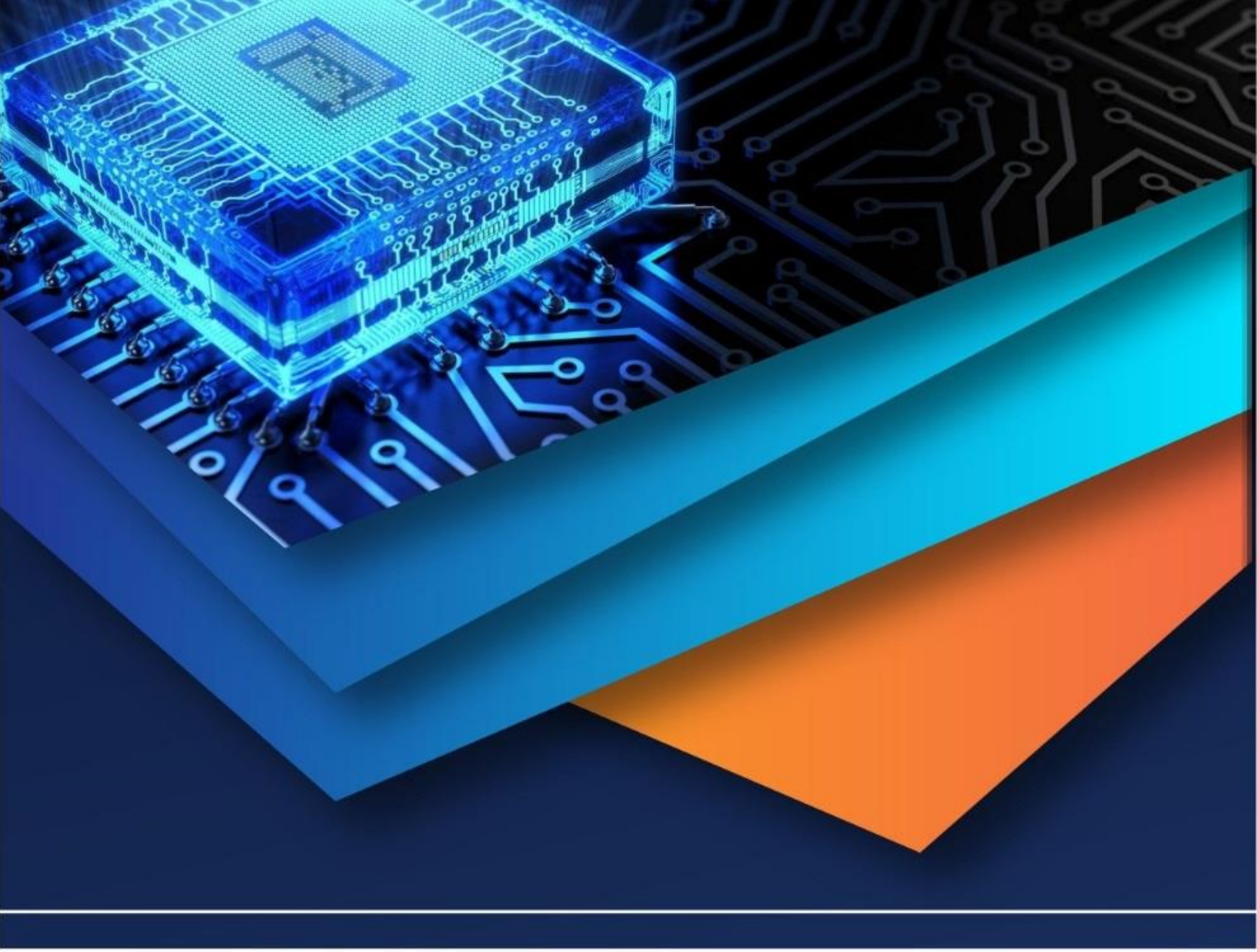
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