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Bioefficacy of Selected Botanicals against Xanthomonas-Induced Bacterial Spot in *Solanum lycopersicum* L

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Abstract: Tomato bacterial spot, caused by Xanthomonas vesicatoria, is a destructive disease that reduces yield and quality. In this study, leaf extracts of Psidium guajava, Azadirachta indica, Ricinus communis, and Lantana camara were evaluated for antimicrobial activity at concentrations ranging from 25–100 ug/ml. The assays revealed dose-dependent inhibition, with P. guajava showing the strongest effect (zone of inhibition up to 22 mm, 86.3% growth suppression at 100 ug/ml). A. indica exhibited moderate activity (15 mm, 68.1%), while R. communis showed steady inhibition (12 mm, 54.5%). L. camara was inactive at lower concentrations but achieved 11.5 mm and 51.1% inhibition at 100 ug/ml. phytochemical profiling indicated that tannins, terpenoids, saponins, and anthraquinones contribute to the observed antimicrobial potency. These findings highlight Psidium guajava leaves extract as the most promising bio-control candidate, with Neem, Castor, and Lantana serving as complementary agents. Collectively, plant-derived phytochemicals offer sustainable alternatives to copper-based bactericides, combining direct pathogen suppression with host defence induction.
Keywords: Xanthomonas, bacterial leafspot, plant extract, phytochemicals, antimicrobial activity, sustainable agriculture.

I. INTRODUCTION

Tomato ranks second after potato in global vegetable production. India is the world's second-largest producer, cultivating approximately 737 thousand hectares and yielding 19,468 thousand metric tonnes annually (HSG, Govt. India, 2025). As one of the most widely consumed vegetable crops, the tomato holds immense economic and nutritional importance. However, its productivity is severely constrained by bacterial and fungal diseases that reduce both yield and quality, among these, bacterial spot caused by Xanthomonas campestris pv. Vesicatoriais particularly destructive. The disease manifests as small, dark lesions on leaves, stems, and fruits, which enlarge over time, leading to defoliation and poor-quality fruit. In warm, humid climates, bacterial spot spreads rapidly and can cause substantial yield losses. Traditionally, bacterial diseases in tomato have been managed through chemical pesticides (Fiorilli et al., 1998). Yet, indiscriminate pesticide use has created significant health and environmental concerns, as residues infiltrate soil, water, and air, ultimately entering the food chain and posing risks to human health.The World Health Organization reports that hazardous agricultural chemicals poison thousands annually in developing nations. In India, 35,000–40,000 tonnes of toxic pesticides are applied each year, contributing to serious health risks such as cancer and sterility. Overreliance on chemical bactericides also contaminates the environment, eliminates beneficial microbes, and fosters the emergence of resistant strains. These concerns highlight the urgent need for safer alternatives. Plant-derived compounds, rich in antimicrobial secondary metabolites, offer promising eco-friendly solutions, and screening plant extracts for antibacterial activity is essential for developing natural disease-control agents (Kumar Bhardwaj et al., 2009). Biopesticides, derived from microbes and botanicals, have gained prominence as sustainable tools that effectively suppress pathogens while minimizing harm to non-target organisms (Saha et al., 2025). Among phytopathogens, Xanthomonas campestris pv. Vesicatoria,the causal agent of bacterial spot in tomato,remains particularly destructive, spreading via contaminated seeds and crop residues. Although copper-based bactericides are widely used, resistance and environmental concerns necessitate alternative strategies (Silva et al., 2022). Given tomato's (Lycopersicon esculentum L.) global economic and nutritional importance, sustainable disease management is critical to safeguard yield and food security.

Bacterial leaf spot, caused by Xanthomonas vesicatoria, is among the most destructive tomato diseases. This rod-shaped, gram-negative bacterium spreads through contaminated practices, wind, rain splashes, and infected seeds, producing water-soaked lesions that turn black and necrotic.

Severe infections lead to defoliation, bloom drop, and reduced fruit quality, resulting in major financial losses (Ahmad & Ahmad, 2022)—similarly, bacterial spot caused by Xanthomonas campestris pv. Vesicatoria is a seed-borne disease that thrives in warm, humid conditions, reducing germination, plant growth, and productivity (Misrak et al., 2013).

Guava (Psidium guajava L.) leaves, long used in traditional medicine, possess antibacterial, antioxidant, and anti-inflammatory properties, making their extracts promising candidates for eco-friendly disease control (Metwally et al., 2010). Given the tomato's global economic and nutritional importance, early and accurate disease detection is critical. Conventional visual inspection methods are often slow and error-prone, underscoring the need for reliable, sustainable approaches to safeguard yield and quality.

Recent advances in deep learning and computer vision have enabled autonomous plant disease identification. Convolutional Neural Networks (CNNs) are particularly effective for classifying leaf images, allowing rapid and precise distinction between healthy and diseased tomato plants, thereby supporting early diagnosis and sustainable crop management (Gupt et al., 2025).Beyond tomato, several crops face devastating bacterial diseases. Arecanut (Areca catechu L.) suffers from bacterial leaf stripe caused by Xanthomonas campestris pv. Arecae, which damages seedlings and can lead to blight and mortality. Pathogen diversity complicates [1]resistance strategies, making variability studies essential (Chanu et al., 2025). Citrus canker, caused by Xanthomonas axonopodis pv. Citri is widespread in tropical and subtropical regions, reducing yield and fruit quality, with losses exceeding 50% in severe cases. Although copper-based bactericides are used, resistance and persistence demand alternative eco-friendly solutions, including plant extracts with proven antimicrobial activity (Negi et al., 2015; Sharma, 2016).Plant diseases remain a major constraint in both conventional and organic farming, underscoring the need for sustainable detection and control strategies to reduce financial losses and protect global food security.Crop yield and development are severely affected by bacterial and fungal diseases. Chemical fungicides, which disrupt cell membranes or metabolic processes, are commonly used for management, but their excessive application pollutes the environment and harms human, soil, and water health. This has created a demand for sustainable alternatives. Plant-derived natural compounds, with proven antiviral, antifungal, and antibacterial properties, are increasingly explored as eco-friendly disease-control agents (Hada et al., 2014).Tomato bacterial spot, caused by Xanthomonas campestris pv. Vesicatoria remains a major threat to yield and quality. Overreliance on chemical bactericides has led to environmental concerns and resistant strains, highlighting the need for eco-friendly alternatives.

Plant-derived extracts offer promising biocontrol potential due to their rich secondary metabolites with antimicrobial activity. Leaves of Psidium guajava, Azadirachta indica, Ricinus communis, Curcuma longa, and Lantana camara have been reported to possess antibacterial, antioxidant, and anti-inflammatory properties (Chandra et al. 2010). Evaluating these extracts against Xanthomonas can provide sustainable strategies for suppressing tomato spot disease under natural conditions, reducing dependence on synthetic chemicals while safeguarding crop productivity and food security.

Evaluating plant extracts such as Psidium guajava, Azadirachtaindica, Ricinuscommunis, and Lantana camara againstXanthomonascampestrispv.vesicatory offers promising, sustainable strategies for suppressing tomato bacterial spot under natural conditions. Such biocontrol approaches reduce dependence on synthetic chemicals, mitigate environmental and health risks, and safeguard crop productivity and food security (Metwally et al., 2010; Kumar Bhardwaj et al., 2009; Silva et al., 2022).

II. MATERIALS AND METHOD

A. Collection of plant material

Psidium guajava, Azadirachta indica, Ricinus communis, and Lantana camara leaves were collected during August (2025) from the rural area, Rahata. Identified by the Botanical department in PVP College, Loni. The Leaves were washed in running tap water, air-dried in shade, and then homogenized to make a fine powder. This powder was packed in air-tight polythene bags until further use.

B. Plant extract preparation

To prepare the plant extract stock, 50 g each of Psidium guajava, Azadirachta indica, Ricinus communis, and Lantana camara leaves were taken, which were cut into small pieces and boiled with 100 ml of sterile distilled water at 100°C for 5-10 minutes. After boiling, the mixture was filtered through Whatman filter paper to obtain the crude extract. The filtrate was then stored at 4°C as a stock solution for further experimentation (Manik et al., 2020).The plant part was washed thoroughly with running tap water, thenair-driedin the shade, and the plant material was ground in a mixer. The powder was kept in plastic bags with labels. The plant extract was prepared by soxhlet extraction method. About 50 gm of powdered plant material was uniformly packed into a thimble and extracted with 250 mL of double-distilled water. After that, the extract was taken in a beaker and kept on hot air oven till all the solvent (aqueous) had evaporated. The dried extract was kept in a small bottle for future use.

C. Qualitative Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by using the following standard methods (Kokate and Purohit, 2015; Sharma et al., 2025).

Test for alkaloids:

The crude extract was mixed with dilute HCl and placed in the water bath for five minutes, and then 1-2 ml of filtrate was filtered, and 2 ml of Wagner'sreagent was added. The reddish-brown color of the precipitate indicated the presence of alkaloids.

Test for Phenolic:

The extract was dissolved in distilled water, and then 2 ml of 5% $FeCl_3$ solution was added. The formation of violet color or blue-green color indicated the presence of phenolic compounds.

Test for Glycosides:

The extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of ferric chloride. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interphase indicated the presence of glycosides.

Test for Saponins:

A test tube containing the extract and five to ten milliliters of distilled water was shaken briskly. The development of steady foam suggested the presence of saponins.

Test for Terpenoids:

2 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. The presence of terpenoids is indicated by the formation of a reddish-brown tint at the interface.

Test for Tannin:

The extract was mixed with 2 to 3 mL of 2% solution of $FeCl_3$.A blue-green or black color indicated the presence of tannins.

Test for flavonoids:

The crude extract was mixed with fragments of magnesium ribbon and concentrated hydrochloric acid; the pink color appeared after a few minutes, which indicated the presence of flavonoids.

Test for Anthraquinones:

2 ml of extract few drops of 10% ammonia solution were added, and the appearance of pink color precipitate indicates the presence of anthraquinones.

D. Pathogenic Isolates

Pathogenic bacterial isolates of Xanthomonas vesicatoria were obtained from the culture collection of previously identified and preserved strains maintained at the Department of Plant Pathology, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra, India. These cultures had been authenticated and preserved under controlled conditions to ensure their stability and reliability for experimental use. The isolates were revived on nutrient agar medium and maintained under aseptic conditions before pathogenicity testing. To confirm the pathogenic nature of the isolates, standard procedures described by Abo-Elyousr and El-Hendawy (2008).

E. In vitro evaluation of plant extracts against the pathogen

The freshly prepared nutrient agar plates (NA) were allowed to dry for approximately 15 minutes in order to eliminate any surface moisture. The NA-petri plates were inoculated with the test micro-organism using aseptic techniques to ensure uniform distribution. A sterile forceps, the paper discs containing various concentrations of plant extract nanoparticles were carefully arranged in a radial pattern and gently pressed onto the surface that had been inoculated. Each disc was placed with enough space between them. Control discs containing the standard antibiotics Gentamicin and negative control discs with the DMSO. The NA-plates were placed in the incubator and left to incubate for 24 to 36 Hrs at a temperature of 370 °C (Thiruvengadam and Bansod, 2020).

III. RESULT AND DISCUSSION

1) Qualitative Phytochemical Analysis:

The result summarizes the presence (+) or absence (–) of major phytochemical groups in Psidium guajava, Lantana camara, Azadirachta indica, and Ricinus communis. The universal occurrence of alkaloids, phenols, terpenoids, and flavonoids across all four plants highlights their strong baseline antimicrobial potential, while distinct compounds such as tannins (unique to guava) and anthraquinones (unique to castor) indicate specialized strengths for biocontrol applications.

Table 1. Phytochemical Screening of Selected Plant Extracts

Sr.No.	Test	P. guajava	L.camara	A. indica	R. communis
	Alkaloids	+	+	+	+
	Phenols	+	+	+	+
	Glycosides	+	-	-	+
	Saponins	+	+	+	-
	Terpenoids	+	+	+	+
	Tannin	+	-	-	-
	Flavonoids	+	+	+	+
	Anthraquinone	-	-	-	+

The phytochemical screening of Psidium guajava, Lantana camara, Azadirachta indica, and Ricinus communis revealed a rich diversity of bioactive compounds relevant for biocontrol of tomato bacterial spot. All four species contained alkaloids, phenols, terpenoids, and flavonoids, which provide a strong baseline of antimicrobial activity through mechanisms such as disrupting bacterial membranes, interfering with protein synthesis, and reducing quorum sensing.

Distinct profiles highlight individual strengths: P. guajava is unique for its tannins, enhancing both pathogen suppression and host defense induction; L. camara is dominated by saponins and terpenoids, effective for rapid bacterial lysis; A. indica (neem) is terpenoid-rich, reinforcing its broad-spectrum biocontrol reputation; and R. communis stands out for anthraquinones, potent antimicrobials requiring careful dose management.

Together, these plants offer complementary strategies,direct inhibition of Xanthomonas and induction of tomato defence responses,making them promising candidates for sustainable, eco-friendly management of bacterial spot.

2) Antimicrobial Activity of Plant Extract against Xanthomonas vesicatoria

The antimicrobial assay showed that all tested plant leaf extracts inhibited the growth of Xanthomonas vesicatoria. All plant leaf extracts showed the strongest and moderate inhibition, making it the most promising biocontrol candidate.

Table 2. Comparative antibacterial efficacy of selected plant leaf extracts against *Xanthomonasvesicatoria*

Sr.No.	Sample	Concentration (ug/ml)	Zone of Inhibition(mm)	% Growth Inhibition
1.	P. guajava leaves extract	Control	0	<5.2%
		25	10	45.5%
		50	20	81.8%
		75	21	84.0%
		100	22	86.3%
2.	A. indica leaves extract	Control	0	<5.2%%
		25	7	31.8%
		50	10	45.5%
		75	13	59.0%
		100	15	68.1%
3.	L.camara leaves extract	Control	0	<5.2%
		25	0	<5.2%
		50	0	<5.2%
		75	8	36.3%
		100	11.5	51.1%
4.	R.communis leaves extract	Control	0	<5.2%
		25	5	22.7%
		50	10	45.5%
		75	11	50.0%
		100	12	54.5%

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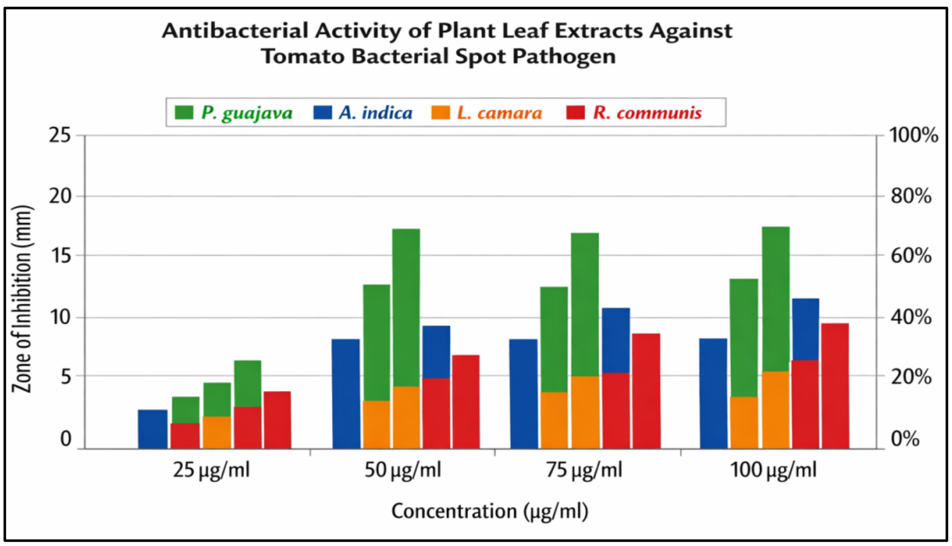
The antibacterial assay of leaf extracts from Psidium guajava, Azadirachta indica, Lantana camara, and Ricinus communis against Xanthomonas spp. demonstrated clear differences in efficacy, reflecting the phytochemical diversity of these plants. Among the tested extracts, P. guajava showed the most pronounced activity, with inhibition zones increasing from 10 mm at 25 ug/ml to 22 mm at 100 ug/ml, corresponding to growth inhibition levels above 80%. This strong dose-dependent response highlights guava's rich composition of phenols, tannins, and flavonoids, which likely act synergistically to suppress bacterial growth and enhance host defense mechanisms.

Neem (A. indica) exhibited moderate activity, with inhibition zones ranging from 7 mm at 25 ug/ml to 15 mm at 100 ug/ml, achieving nearly 70% growth inhibition at the highest concentration. The presence of terpenoids such as azadirachtin, along with alkaloids and saponins, supports neem's reputation as a broad-spectrum biocontrol agent, though its efficacy was lower than that of guava under the tested conditions.

Lantana (L. camara) showed weak activity at lower concentrations, with no inhibition observed at 25–50 ug/ml. However, at higher doses (75–100 ug/ml), inhibition zones of 8–11.5 mm were recorded, corresponding to ~36–51% growth inhibition. This suggests that lantana's saponins and terpenoids contribute to antimicrobial activity, but only at elevated concentrations, limiting their standalone effectiveness.

Castor (R. communis) displayed moderate inhibition, with zones ranging from 5 mm at 25 ug/ml to 12 mm at 100 ug/ml, corresponding to ~23–55% growth inhibition. Its activity is likely attributed to anthraquinones and glycosides, which provide oxidative and antimicrobial effects, though the absence of saponins and tannins may reduce its capacity to induce host defenses compared to guava or neem.



The results clearly rank the extracts in effectiveness. Guava showed the strongest inhibition, making it the most promising bio-control candidate, while Neem provided moderate suppression. Castor and Lantana, though less potent, may complement in synergistic formulations. Overall, these findings highlight plant-derived phytochemicals as sustainable alternatives to copper bactericides, offering both pathogen inhibition and host defence induction.

IV. CONCLUSION

The antimicrobial assays confirmed that leaf extracts of Psidium guajava, Azadirachta indica, Ricinus communis, and Lantana camara inhibited the growth of Xanthomonas vesicatoria, though with varying potency. P. guajava exhibited the strongest activity, establishing it as a promising biocontrol candidate, while neem provided moderate suppression. Castor and lantana, though less potent, may serve as complementary agents in synergistic formulations. Phytochemical profiling supports these findings, with guava and neem enriched in tannins and terpenoids that can suppress the pathogen and enhance host defense responses, while lantana and castor contribute saponin- and anthraquinone-mediated antimicrobial effects. Collectively, these findings highlight the potential of plant-derived phytochemicals as sustainable alternatives to copper-based bactericides, offering both direct pathogen inhibition and host defence induction, thereby aligning with eco-friendly agricultural practices.

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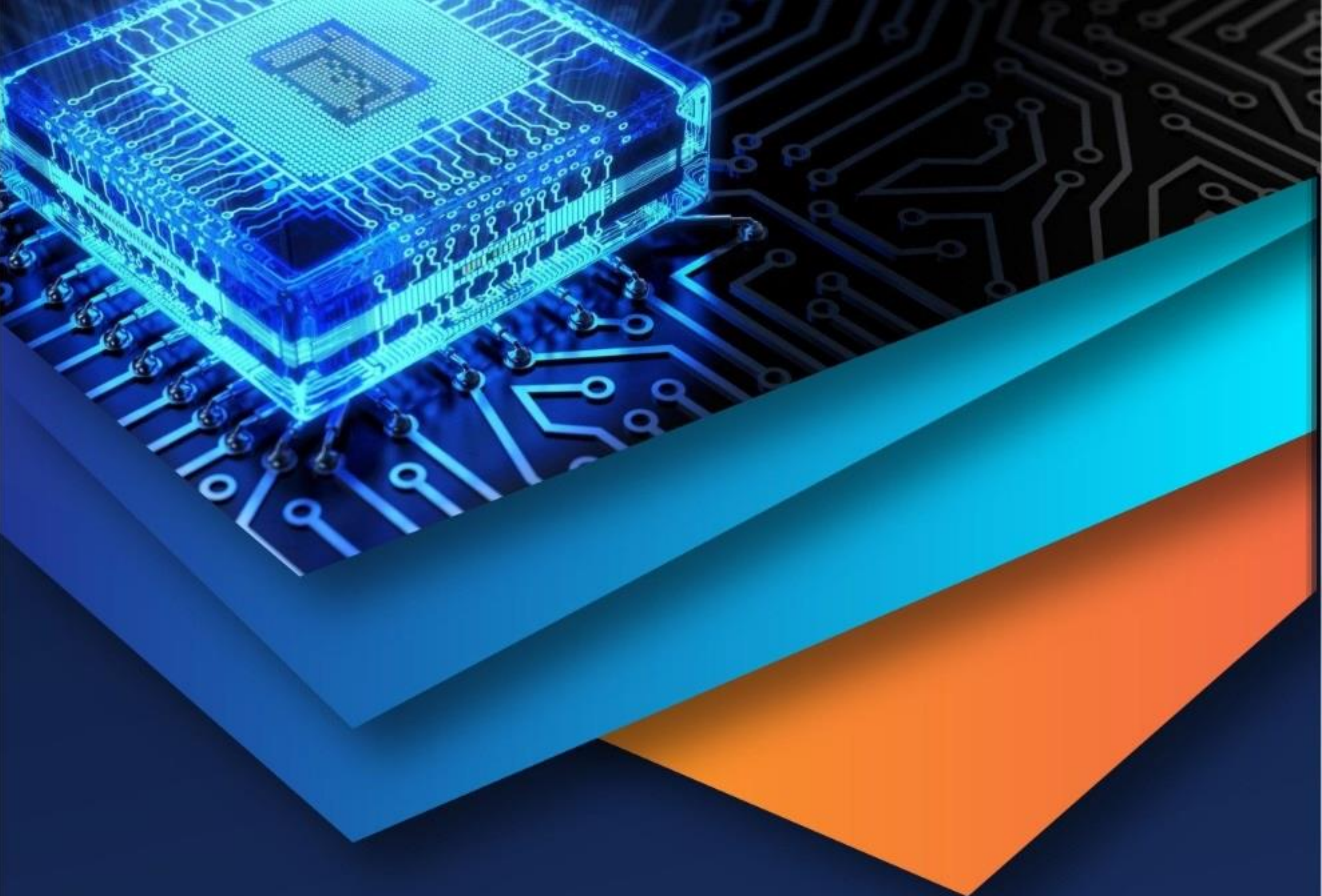

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