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Antioxidant and Antibacterial Activity of Cinnamaldehyde Derivative

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Abstract: ***This study reports the antibacterial and antioxidant properties of cinnamaldehyde derivatives. DPPH, Superoxide Anion Radical, Hydroxy Radical, and Nitric Oxide Radical Scavenging Activities were used to assess the test compound's antioxidant capacity. The well diffusion method was used to examine the antibacterial efficiency against both Gram positive and Gram-negative microorganisms. Both Gram positive and Gram-negative bacterial strains responded favourably to the antibacterial effects of cinnamaldehyde derivatives. The study revealed the test substance possesses strong antibacterial and radical scavenging abilities.***
Keywords: Cinnamaldehyde, Antioxidant, Antimicrobial

I. INTRODUCTION

Free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), are created in biological systems as a result of oxygen reduction or the breakdown of xenobiotics. These radicals are unstable and can damage key biomolecules like lipid membranes or membrane proteins, leading to decreased fluidity, loss of enzymes, receptor activity and damage to membrane proteins. Cells have a variety of defence mechanisms to reduce free radical formation or limit its damaging effects. However, these mechanisms are not always sufficient to keep the balance in favour of free radicals' generation. Oxidative stress is a problem that has been studied extensively for many years, and there are many compounds that can protect against it. Phytochemicals have been shown to scavenge free radicals and inhibit lipid peroxidation, which is a major concern seen in the food industry.

A variety of microorganisms can lead to food spoilage, so it is important to know which ones are present in food products. At first, synthetic chemicals were used to prevent oxidation and microbial contamination of dietary components.

But people are becoming more concerned about the side effects of synthetic compounds and they are in need for a safer material to prevent and control these pathogenic microorganisms that contaminate food and other natural products.

Phytochemicals have the potential to modify human metabolism in a way that is beneficial for the prevention of chronic and degenerative diseases that is commonly seen nowadays. In the present-day spices and aromatic plant materials are used to enhance the flavour and fragrance qualities of food materials in the food industry.

In addition, some natural products are also used as traditional medicine. Spices are a good source of natural antioxidants, and they play a huge role in preventing the growth of bacterial species. Cinnamon's antioxidant activity is due to the components present in them, like cinnamaldehyde, eugenol, cinnamic acid and cineol. Cinnamaldehyde is a member of the phenylpropanoid class of phytochemicals. This study reports the antioxidant potential and antibacterial activities of cinnamaldehyde derivative.

II. MATERIALS AND METHODS

A. DPPH (1,1-diphenyl-2-Picryl Hydraxyl) Radical Scavenging Assay

A dark room was used to measure spectrophotometrically the radical-scavenging capacity of cinnamaldehyde against DPPH. A hydrogen radical or an electron can be accepted by DPPH, to transform it into a stable diamagnetic molecule. DPPH reacts with an antioxidant molecule that can give hydrogen in order to get reduced. Measurements were made of the colour change (from deep violet to blue). According to the quantity and type of radical scavengers present in the sample, a certain degree of blue colour develops.

1) Reagents

a) 1,1-diphenyl-2-picryl hydrazine.

2) Procedure

In order to make 3ml of water, 1ml of DPPH and 1ml of various amounts of cinnamaldehyde derivative were added. The blue colour developed was measured at 517 nm using vitamin C as the standard.

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

B. Nitric Oxide Scavenging Activity

Cinnamaldehyde's capacity to scavenge nitric oxide was evaluated. NEDD can be used to calculate the amount of nitrite ions produced as a result of the reaction between oxygen and nitric oxide produced from sodium nitroprusside in an aqueous solution at physiological PH. Nitric oxide scavenging works against oxygen to reduce the formation of nitrite ions.

1) Reagents

a) Sodium nitroprusside
b) Sulphanilic acid
c) Glacial Acetic Acid
d) NEDD (Naphthyl Ethylene Diamine Dihydrochloride)
e) PBS

2) Procedure

Various quantities of the test extract were combined with 2.0 ml of 10 mM sodium nitroprusside in phosphate buffered saline, and the mixture was incubated at 25oC for 150 minutes. The incubated solution was diluted to 0.5 ml and combined with 1 ml of sulphanilic acid. When the nitrite was diazotized with sulphanilic acid and then coupled with NEDD (Naphthyl Ethylene Diamine Dihydrochloride), the chromophore's absorbance was measured at 540 nm. Standardization was done using vitamin C.

C. Superoxide Anion Radical Scavenging Assay

Measurement of superoxide anion scavenging activity was carried out based on this method.

1) Reagents

a) NBT Solution:
100µM of NBT was prepared with 100ml phosphate buffer (P^H8)
b) NADH
468 µM of NADH was dissolved in 100ml phosphate buffer (P^H8).
c) Sample preparation:
100, 200, 400, and 800 µg in 1ml of DMSO
d) PMS (Phenazine Methosulfate)
60 µM of PMS was dissolved in 100 ml of Phosphate Buffer

2) Procedure

1ml of the test sample solution was combined with 1ml of the NBT solution and 1ml of the NADH solution. This mixture was thoroughly mixed with 100 µl of PMS, and it was then incubated at 30 °C for 15 minutes. The reagent mixture and without sample were used to perform a control. At 560 nm, absorbance was spectrophotometrically measured. A reference standard was used, which was vitamin C.

$$\text{Superoxide anion scavenging activity (\%)} = ((Ao - A1) / Ao) \times 100$$

Where Ao is the absorbance of the control, and A_1 is the absorbance of the sample.

D. Hydroxyl Radical Scavenging Activity

The scavenging activity of the cinnamaldehyde on hydroxyl radical by this method

1) Reagents

a) EDTA solution 37mg of EDTA in 100 ml of KPO_4 buffer

b) $FeCl_3$ (500 µM) solution
c) Deoxy Ribose (15Mm) in 20ml of KPO_4 Buffer
d) TBA (1%) in 0.05N NaOH
e) TCA (28%) in 100 ml of Water
f) Hydrogen Peroxide

2) Procedure

Various extract concentrations (100, 200, 400, and 800 g/ml of DMSO) were combined with 0.2 ml of FeCl3 solution, 0.1 ml of EDTA solution (1 mM), and 1.0 ml of hydrogen peroxide (H2O2). Deoxy ribose was added to the mixture to start the reaction, which was then left to sit for 60 minutes at room temperature. After incubation, the reaction was stopped by adding 1.0 ml of TCA. The TBA solution was diluted to 1 ml and heated to 95 °C for 30 minutes. The reaction mixture was centrifuged for 15 minutes at 5319 rpm after being cooled with ice. At 535 nm, the intensity of the produced colour was determined spectrophotometrically. The reference standard used was vitamin C.

$$HRSA\ (\%) = (Ao - A1) / Ao * 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

E. Antimicrobial activity assessment

Antimicrobial tests: Antimicrobial activity of the cinnamaldehyde was investigated by using the Disc Diffusion assay.
Reference and control: Ampicillin were chosen as the reference antimicrobial drug for all bacterial species including E. coli. The control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganisms mixed in a 1:1 ratio.

F. Collection of clinical pathogens

The Bacterial Pathogens *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae* and *Escherichia coli were* obtained from RMMCH (Rajah Muthaih Medical College and Hospital) Chidambaram, Tamil Nadu.

G. Procedure - Well diffusion method

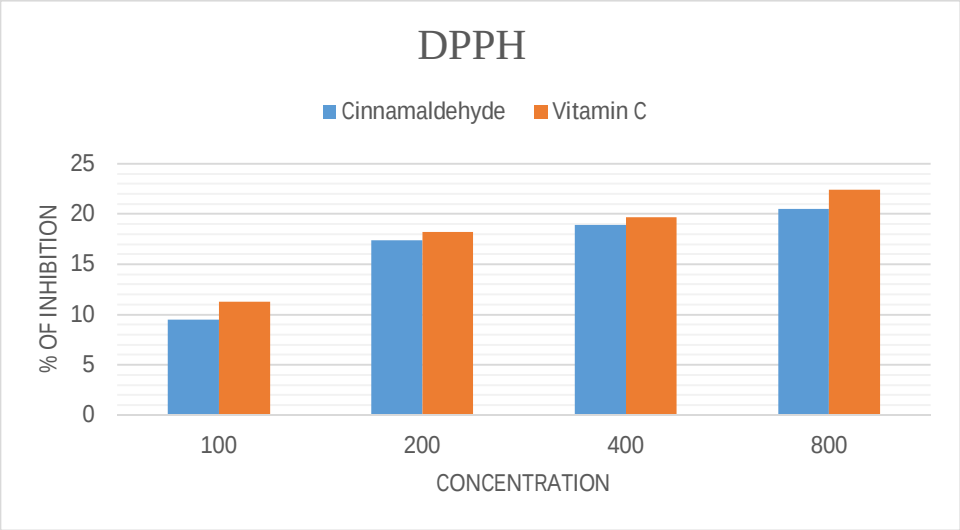
By using the well diffusion method, the antibacterial activity of the active fractions was evaluated against bacterial pathogens. This was done by inoculating bacterial pathogens in nutrient broth and allowing them to grow for 12 hours before performing an antibacterial assay. On the Muller Hinton agar plates, every bacterial strain was dispersed separately. Using a cork borer, wells measuring 6 mm were drilled into the plates. The wells were filled with the various concentrations of 50 l, 75 l, and 100 l of the compounds before being incubated for 24 hours at 37 °C (Saadoun and Muhana, 2008). The assay was performed three times. After the incubation period was over, the zone of inhibition was measured in millimetres. All the data were statistically analysed.

III. RESULTS AND DISCUSSIONS

A single assay approach is insufficient to study the antioxidant capacity of endogenous substances. The assay concept and testing procedure for various antioxidant tests also varies. For instance, some techniques make use of DPPH or other organic radical producers. They differ from one another in terms of how long it takes for their chemical processes to result in free radicals through an oxidation reaction. Different antioxidants are taken into account as a control for distinct assay procedures according to the time they require for scavenging because the procedure and experimental circumstances differ for different techniques. Additionally, antioxidants can be either non-polar (like vitamin E) or polar (like phenolic, flavonoids, etc.) in nature, and they can scavenge free radicals via either an electron-donating mechanism or a hydrogen-donating mechanism. As a result, various control antioxidants (such AA) were utilised for various antioxidant assays.

A. DPPH Radical Scavenging Activity

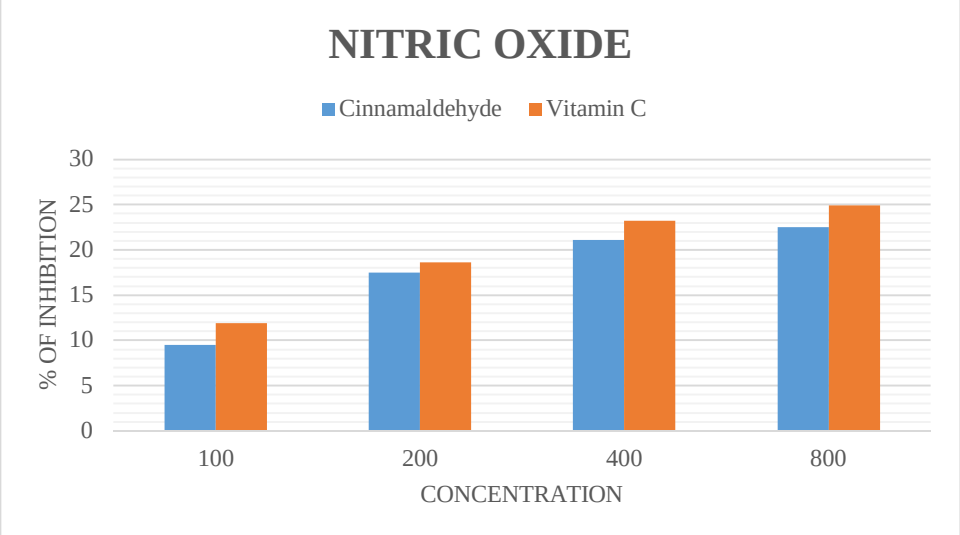
According to Baumann et al. (1979), ability of antioxidant to donate hydrogen is assumed to be the reason for their impact on DPPH. Radical scavenging activities are particularly crucial to prevent the detrimental effect of free radicals in many diseases, including CVDs. Scavenging of DPPH free radicals is a common method for determining the antioxidant activity. In the DPPH assay, the addition of the extract reduces the violet colour of the DPPH solution to a yellow-coloured product. Due of the quick turnaround time for analysis, this approach has been widely utilised to predict antioxidant activity.



The dose-response curve of cinnamaldehyde's DPPH radical scavenging activity is depicted in this picture. Our findings showed that cinnamaldehyde has the same free radical-scavenging properties as standard vitamin C. It was clear that cinnamaldehyde had proton-donating ability and this might serve as free radical inhibitors or scavengers, possibly acting as major antioxidants, even if its DPPH radical scavenging skills were slightly lower than those of vitamin c. The amount of the cinnamaldehyde extract that inhibited the DPPH radical was 9.5, 17.4, 18.9, and 20.5% at 100, 200, 400, and 800 g/ml. The findings of this study indicate that the ability to donate hydrogen or to transfer electrons can enhance the ability to scavenge cinnamaldehyde radicals.

B. Nitric Oxide Scavenging Assay

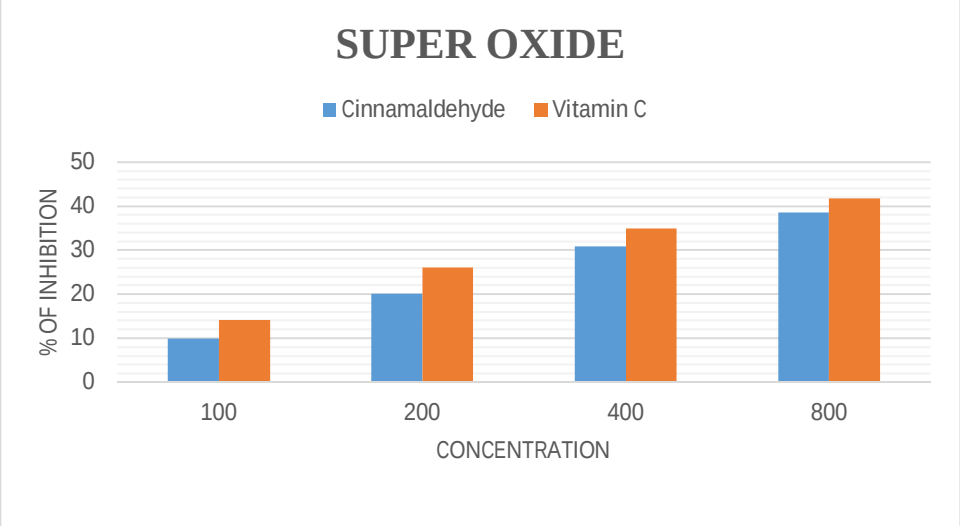
Nitric oxide is a strong pleiotropic modulator of many physiological processes. It is a diffusible free radical that functions in a variety of biological systems as an effector molecule. Due to its extreme instability, it interacts with oxygen molecules to form stable nitrate and nitrite that may be measured using the NEDD reagent. The amount of nitrous acid, which is detectable at 546 nm, will decrease in the presence of a scavenging test agent. The below graph illustrates a large clearance of NO radical caused by the scavenging activity of cinnamaldehyde in a dose-dependent manner.



above graph plots percentage-free radical scavenging against the various cinnamaldehyde concentrations. Cinnamaldehyde had the highest percentage of inhibition at 22.5%, and its ability to scavenge NO rose as its concentration did as well. When cinnamaldehyde and vitamin c are compared in terms of the percentage of inhibition, cinnamaldehyde's inhibition rate is highly dependent on its concentration, and its percent of inhibition is comparable to vitamin c's.

C. Superoxide Anion

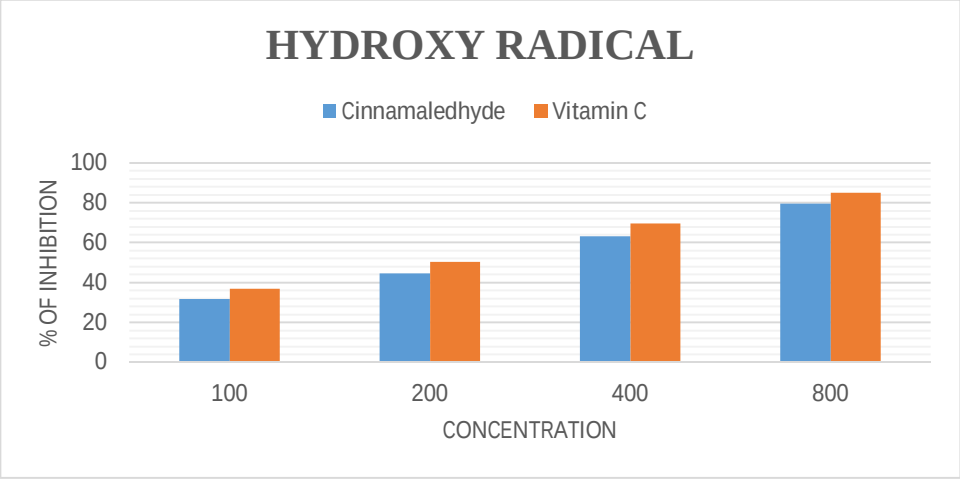
Although superoxide is seen as a relatively inactive oxidant with little chemical reactivity, it can nonetheless produce more hazardous species such singlet oxygen and hydroxyl radicals, which can lead to damaging of lipids. Numerous enzyme systems create these species. Thus, active free radicals that have the ability to react with biological macromolecules and cause tissue damage are considered as the precursors of superoxide anion. NBT will be decreased in this system by superoxide anions produced by the riboflavin/methionine/illuminate system from dissolved oxygen. In this procedure, the yellow dye (NBT2+) is reduced by the superoxide anion to create the blue formazan, which is detected spectrophotometrically at 560 nm. Antioxidants have the ability to prevent the production of blue NBT. Antioxidants cause the absorbance at 560 nm to drop, which shows that superoxide anion consumption has occurred.



The graph above demonstrates how effectively and efficiently cinnamaldehyde inhibits the synthesis of blue formazan in a dose-dependent way. The proportion of inhibitory concentration rises with dose, from 9.9% to 38.5%, at low concentrations of 100 g/ml to high concentrations of 800 g/ml.

D. Hydroxy Radical Scavenging Activity

OH radicals are short lived and can seriously harm other biomolecules and cell membranes. Therefore, OH radical scavenging is required to shield cells from oxidative harm. The Fenton reaction, which includes the transition metal-catalysed breakdown of hydrogen peroxide to form hydroxyl radicals, is the most significant of the numerous ways that OH radicals can be produced.



E. Effect Of Cinnamaldehyde On Antimicrobial Activity

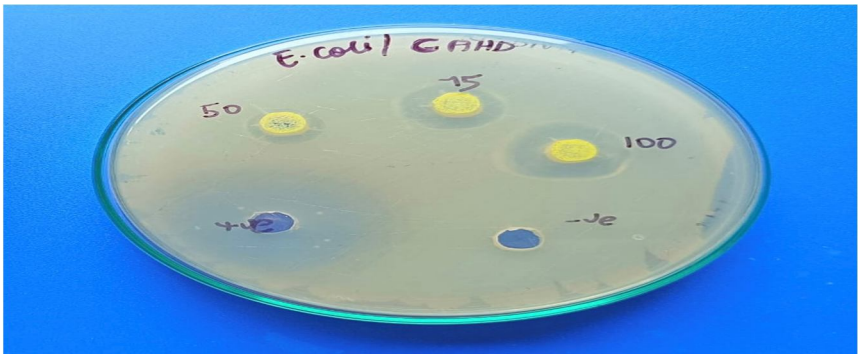
The antibacterial activity of CAHD Sample against bacterial pathogens were tested by a well diffusion method.

IV. RESULT

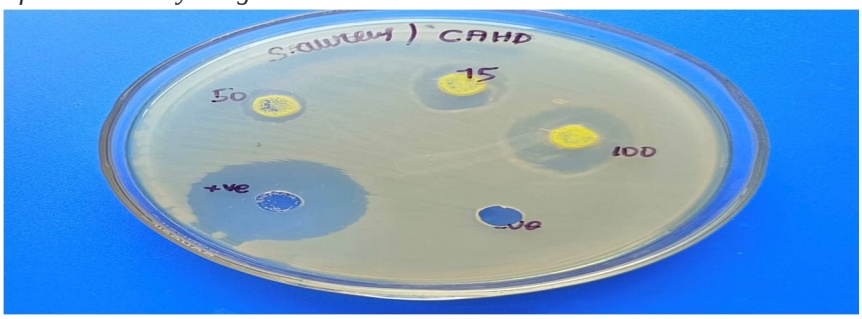
The CAHD Sample revealed that, at the specific concentration of 100 µl, the zone of inhibition against Klebsiella pneumoniae was the largest (26 mm), followed by that against Streptococcus pyogenes (24 mm), Staphylococcus aureus (16 mm), and Escherichia coli (18 mm).

INHIBITION ZONE

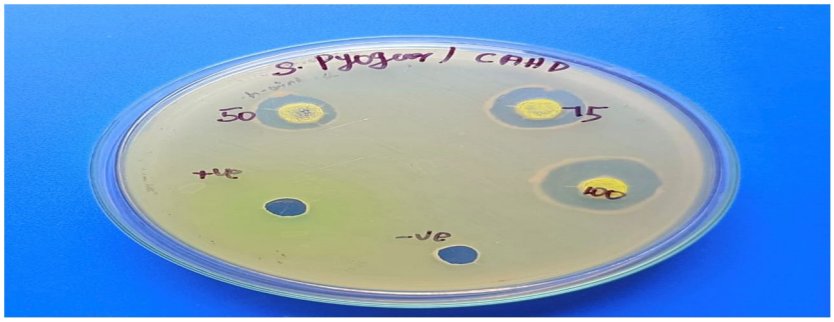
1) *Antibacterial Activity of Cinnamaldehyde Against E Coli*



2) *Antibacterial Activity of Cinnamaldehyde Against S.Aureus*



3) *Antibacterial Activity Of Cinnamaldehyde Against S.Pyogenes*



4) *Antibacterial Activity Of Cinnamaldehyde Against K.Pneumoniae*

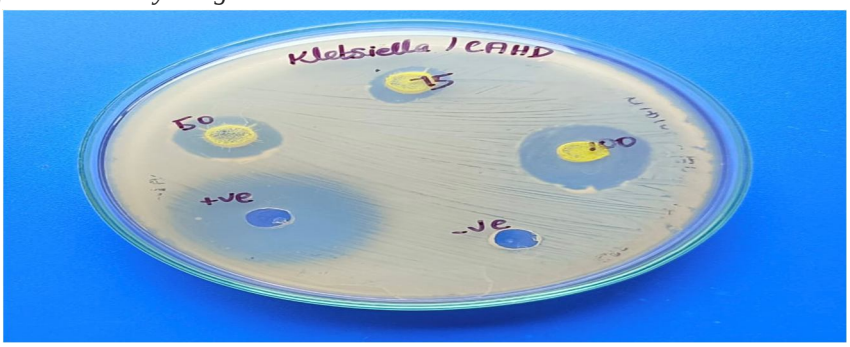


Table Antibacterial activity of CAHD SAMPLE

Bacterial pathogens	Zone of Inhibition				
	50 µl	75 µl	100 µl	Positive	Negative
Staphylococcus aureus	14	16	20	30	-
Streptococcus pyogenes	18	20	24	30	-
Klebsiella pneumonia	20	24	26	32	-
Escherichia coli	12	16	18	32	-

V. CONCLUSION

As per the findings of the present study, cinnamaldehyde was revealed to be an effective antioxidant by conducting a variety of in vitro assays, such as DPPH, hydroxy radical scavenging, nitric oxide, and superoxide anion, when compared to conventional antioxidants like vitamin C. At a concentration of 100 µl, the antibacterial activity of cinnamaldehyde was highly effective against Klebsiella pneumonia (26 mm), Streptococcus pyogenes (24 mm), Staphylococcus aureus (16 mm), and Escherichia coli (18 mm).

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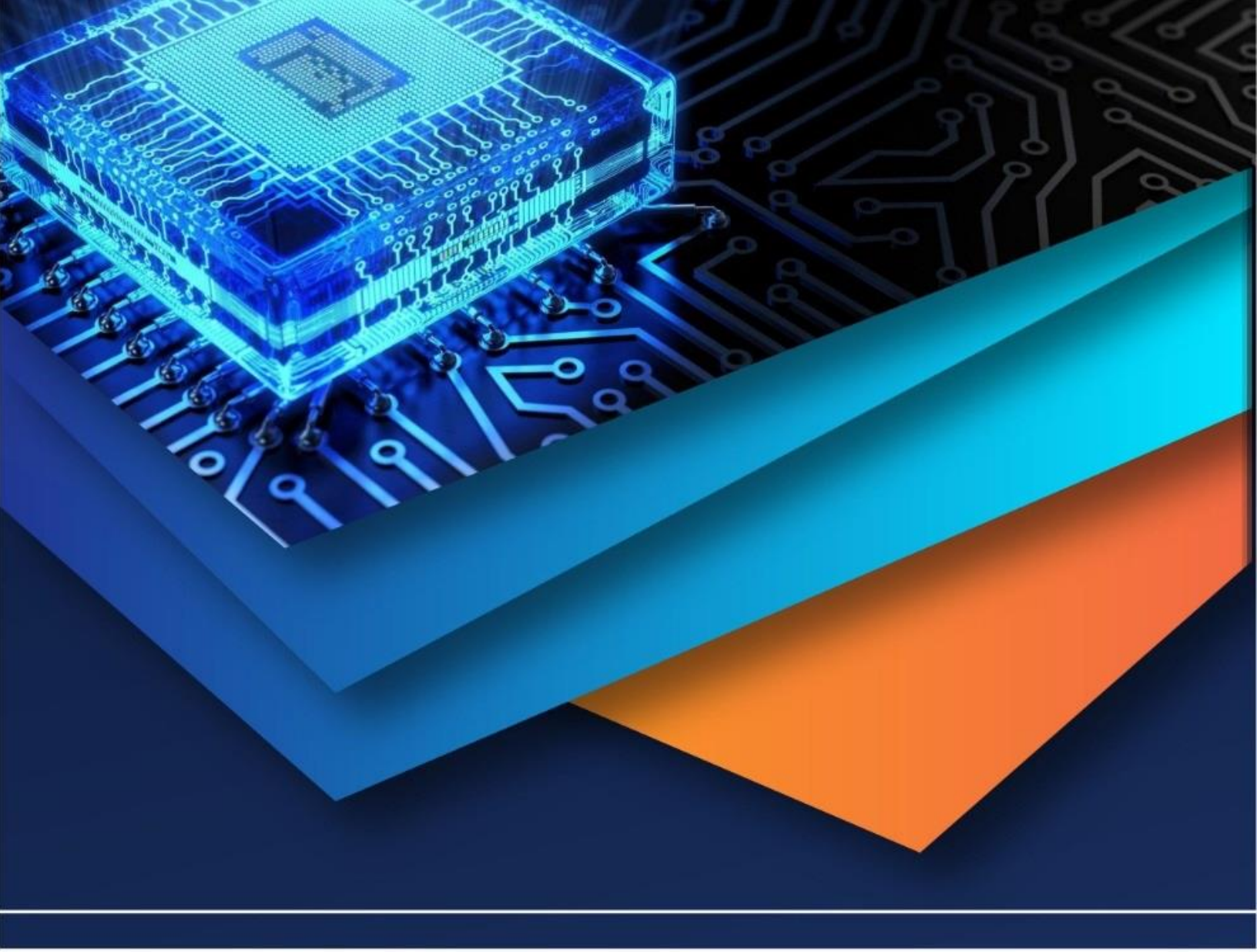

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