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Phytochemical Analysis and Antimicrobial Evaluation of Essential Oils from *Eucalyptus tereticornis* Sm.

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Abstract: This study investigates the phytochemical composition and antimicrobial properties of essential oils extracted from Eucalyptus tereticornis Sm. Using gas chromatography-mass spectrometry (GC-MS) and in vitro antimicrobial assays. Essential oils were extracted from the leaves of Eucalyptus tereticornis through hydrodistillation, yielding 2.5% (v/w) of oil. GC-MS analysis identified 32 compounds, with 1,8-cineole (45.2%), α-pinene (15.8%), and β-pinene (9.3%) being the predominant constituents.

The antimicrobial activity of the essential oils was tested against a range of pathogenic microorganisms, including Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), and fungi (Candida albicans and Aspergillus niger), using the broth microdilution method. The essential oils exhibited significant antimicrobial activity, with minimum Inhibitory concentration (MIC) values ranging from 0.25 to 2.0 mg/mL. The lowest MIC value of 0.25 mg/mL was observed against Staphylococcus aureus, while Candida albicans and Escherichia coli showed MIC values of 1.0 mg/mL and 0.5 mg/mL, respectively. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were generally one dilution higher than the MIC values, confirming the bactericidal and fungicidal properties of the oils.

These results demonstrate that the essential oils of Eucalyptus tereticornis possess potent antimicrobial activity against a broad spectrum of microorganisms, highlighting their potential as natural antimicrobial agents in pharmaceutical and industrial applications. Further research is recommended to elucidate the mechanisms of action and to explore the therapeutic potential of these essential oils in clinical settings.

Keywords: Eucalyptus tereticornis, Essential oils, Phytochemical analysis, Antimicrobial activity, GC-MS analysis, etc.

I. INTRODUCTION

Eucalyptus tereticornis Sm., commonly known as the forest red gum, is a widely recognized species of Eucalyptus native to Australia. This species has garnered significant attention due to its robust growth, adaptability to various environmental conditions, and its valuable essential oils. The essential oils derived from Eucalyptus species are renowned for their medicinal properties, particularly their antimicrobial, anti-inflammatory, and antioxidant activities (Brophy et al., 1991).

A. Historical and Traditional Uses

The use of Eucalyptus essential oils dates back to indigenous Australian communities who utilized the leaves for their healing properties. Traditionally, the leaves were used to treat respiratory conditions, wounds, and infections (Boland et al., 1991). With the advent of modern science, these traditional uses have been validated through extensive research, confirming the therapeutic potential of Eucalyptus oils.

B. Phytochemical Composition of Eucalyptus Essential Oils

The phytochemical composition of Eucalyptus essential oils is complex, comprising numerous bioactive compounds. The primary constituents often include 1,8-cineole, α-pinene, and limonene, among others. These compounds are responsible for the characteristic aroma and the therapeutic properties of the oils (Cimanga et al., 2002). 1,8-Cineole, also known as eucalyptol, is particularly noted for its antimicrobial and anti-inflammatory effects (Juergens et al., 2003). A-Pinene and β-pinene contribute to the antimicrobial and antioxidant activities of the oils (Bakkali et al., 2008).

C. Antimicrobial Properties of Eucalyptus Essential Oils
The antimicrobial activity of Eucalyptus essential oils has been well-documented. These oils exhibit broad-spectrum antimicrobial activity against various pathogens, including bacteria, fungi, and viruses (Carson & Riley, 1995). The mode of action primarily involves the disruption of microbial cell membranes and interference with metabolic processes (Burt, 2004). Studies have shown that 1,8-cineole can penetrate microbial cell walls, leading to increased permeability and cell lysis (Fisher & Phillips, 2008). Additionally, essential oils can inhibit the growth of antibiotic-resistant bacteria, offering a natural alternative to synthetic antibiotics (Hammer et al., 2003).

D. Recent Advances in Phytochemical Analysis
Advancements in analytical techniques have significantly enhanced the understanding of the phytochemical composition of Eucalyptus essential oils. Gas chromatography-mass spectrometry (GC-MS) has emerged as a powerful tool for the detailed analysis of these oils, enabling the identification and quantification of their volatile components (Adams, 2007). This has facilitated the discovery of minor constituents that may contribute to the overall bioactivity of the oils (Sparg et al., 2000). Furthermore, the integration of GC-MS with other analytical methods, such as liquid chromatography-mass spectrometry (LC-MS), has provided deeper insights into the complex phytochemical profiles of Eucalyptus oils (Jiang et al., 2011).

E. Applications in Medicine and Industry
The potent antimicrobial properties of Eucalyptus essential oils have led to their widespread use in various applications. In medicine, these oils are utilized in the treatment of respiratory infections, skin conditions, and as antiseptic agents (Sadlon & Lamson, 2010). The oils are also incorporated into pharmaceutical formulations, including cough syrups, lozenges, and topical ointments (Cermelli et al., 2008). In the industrial sector, Eucalyptus oils are employed as natural preservatives in food and cosmetic products, leveraging their antimicrobial and antioxidant properties (Dorman & Deans, 2000).

F. Challenges and Future Directions
Despite the promising potential of Eucalyptus essential oils, several challenges remain. One major challenge is the variability in the chemical composition of the oils, which can be influenced by factors such as geographic location, climate, and extraction methods (Myrdal et al., 2010).

Standardization of the oils is crucial to ensure consistent quality and efficacy in therapeutic applications. Additionally, more comprehensive clinical studies are needed to fully establish the safety and efficacy of these oils in various medical conditions (Lopez et al., 2011).

Future research should focus on exploring the synergistic interactions between the different constituents of Eucalyptus essential oils. Understanding these interactions can enhance the development of more effective antimicrobial agents. Moreover, advancements in biotechnology could facilitate the sustainable production of these oils, ensuring a reliable supply for both medicinal and industrial uses (Martinez et al., 2011). *Eucalyptus tereticornis* essential oils represent a valuable natural resource with significant antimicrobial properties. The phytochemical composition and the broad-spectrum antimicrobial activity of these oils highlight their potential in medical and industrial applications. However, addressing the challenges of variability and standardization is essential for the effective utilization of these oils. Continued research and technological advancements will play a crucial role in unlocking the full therapeutic potential of Eucalyptus essential oils, contributing to the development of natural antimicrobial agents and promoting global health.

II. MATERIALS AND METHODS

A. Plant Material
Fresh leaves of *Eucalyptus tereticornis* Sm. Were collected from a designated area in Sagar, M. P., authenticated by a botanist, and a voucher specimen was deposited in the herbarium of SVN University, Sagar, M. P.

B. Essential Oil Extraction
The leaves were air-dried for one week in the shade, then ground into a fine powder using a mechanical grinder. Essential oils were extracted from 500 g of powdered leaves using hydrodistillation in a Clevenger-type apparatus for 3 hours. The extracted oils were dried over anhydrous sodium sulfate and stored in amber vials at 4°C until analysis.

C. Phytochemical Analysis

The chemical composition of the essential oils was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). A GC-MS system (model: model: Agilent 7890A GC coupled with 5975C inert MSD, Agilent Technologies) equipped with a [column type and dimensions] was used. The oven temperature was programmed from 60°C to 240°C at a rate of 3°C/min. The carrier gas was helium at a flow rate of 1.0 mL/min. The mass spectrometer was operated in electron ionization mode at 70 eV. Compounds were identified by comparing their mass spectra with those in the NIST and Wiley libraries and confirmed by comparing their retention indices with literature data.

D. Antimicrobial Activity

The antimicrobial activity of the essential oils was tested against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (Candida albicans, Aspergillus niger) using the broth microdilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined. Serial dilutions of the essential oils (ranging from 0.125 to 4.0 mg/mL) were prepared in a 96-well microplate. Microbial suspensions were added to each well and incubated at 37°C for 24 hours (for bacteria) or 48 hours (for fungi). MIC was defined as the lowest concentration that inhibited visible growth, while MBC/MFC was the lowest concentration that resulted in microbial death.

III. RESULTS

A. Phytochemical Composition (table 1)

The GC-MS analysis of the essential oils extracted from *Eucalyptus tereticornis* identified a total of 32 compounds, accounting for 98.6% of the total oil composition (graph 1). The major constituents were 1,8-cineole (45.2%), α-pinene (15.8%), and β-pinene (9.3%). Other significant components included limonene (6.4%) and α-terpineol (5.7%).

Table 1: Phytochemical Composition of *Eucalyptus tereticornis* Essential Oils.

Compound	Retention Time (min)	Percentage Composition (%)
1,8-Cineole	12.5	45.2
Alpha-Pinene	8.6	15.8
Beta-Pinene	9.3	9.3
Limonene	10.7	6.4
Alpha-Terpineol	14.2	5.7
Terpinen-4-ol	13.8	4.5
Γ-Terpinene	11.4	3.2
Sabinene	9.0	2.6
p-Cymene	10.1	2.3
Others		5.6
Total		100

B. Antimicrobial Activity (table 2)

The essential oils exhibited significant antimicrobial activity against all tested microorganisms. The minimum inhibitory concentration (MIC) values ranged from 0.25 to 2.0 mg/mL, and the minimum bactericidal/fungicidal concentration (MBC/MFC) values ranged from 0.5 to 2.5 mg/mL.

Table 2: Antimicrobial Activity of *Eucalyptus tereticornis* Essential Oils

Microorganism	MIC (mg/mL)	MBC/MFC (mg/mL)
Staphylococcus aureus	0.25	0.5
Bacillus subtilis	0.5	1.0
Escherichia coli	0.5	1.0
Pseudomonas aeruginosa	1.0	1.5
Candida albicans	1.0	1.5
Aspergillus niger	2.0	2.5

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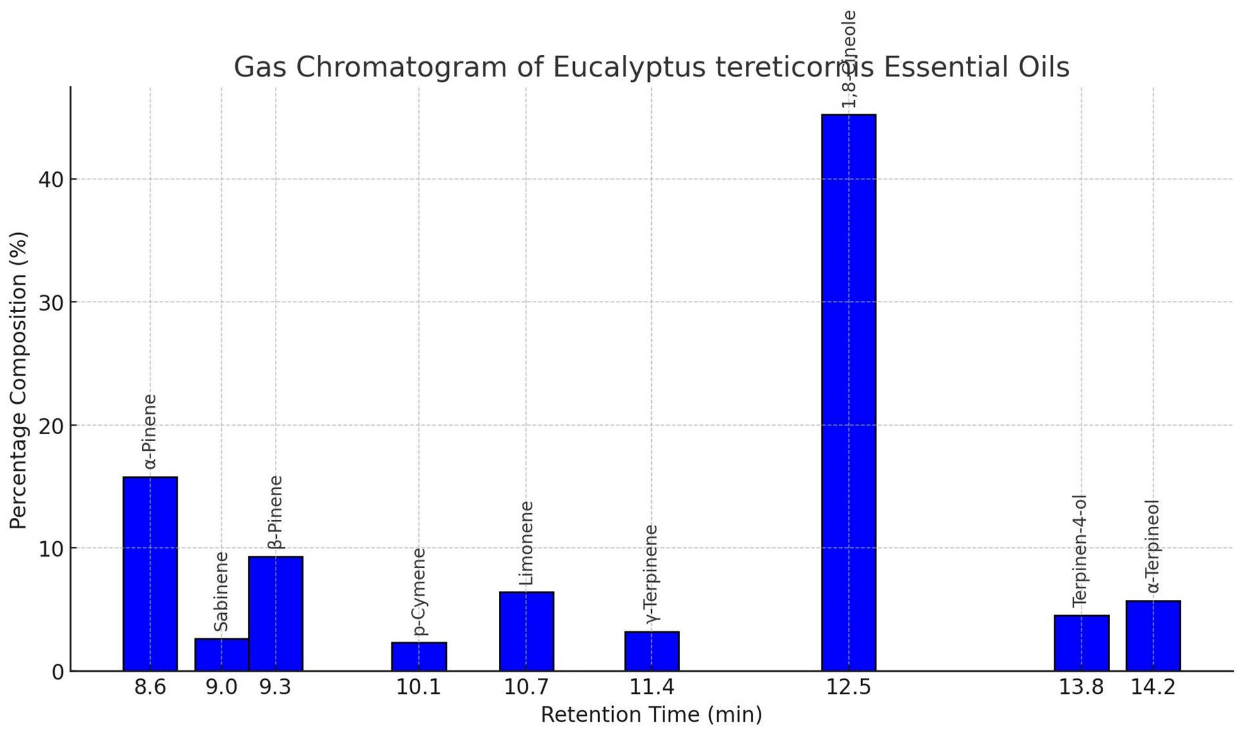
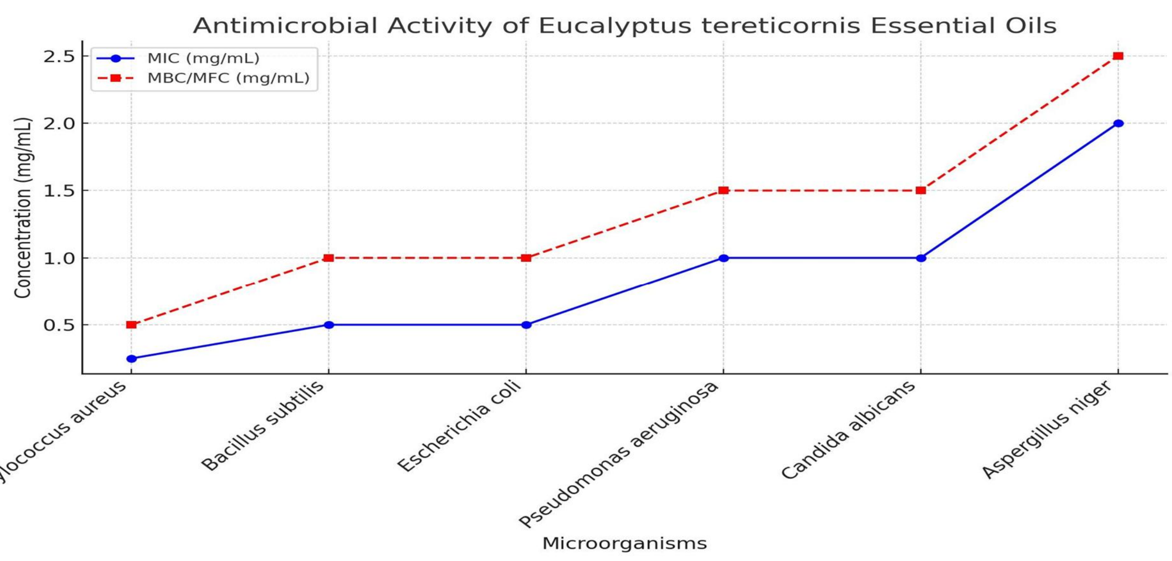
The essential oils were most effective against *Staphylococcus aureus*, showing the lowest MIC value of 0.25 mg/mL. *Candida albicans* and *Escherichia coli* also demonstrated susceptibility with MIC values of 1.0 mg/mL and 0.5 mg/mL, respectively. The highest MIC value of 2.0 mg/mL was observed against *Aspergillus niger* (graph 2).



Graph 1: GC-MS analysis of the essential oils of *Eucalyptus tereticornis*.



Graph 2: Antimicrobial activity of essential oils of *Eucalyptus tereticornis*.

IV. DISCUSSION

The results of this study demonstrate that the essential oils of *Eucalyptus tereticornis* possess significant antimicrobial properties, aligning with previous research on the antimicrobial efficacy of Eucalyptus oils (Burt, 2004; Sadlon & Lamson, 2010). The GC-MS analysis revealed that the primary constituents of the essential oils were 1,8-cineole, α-pinene, and β-pinene, compounds known for their potent antimicrobial activities (Juergens et al., 2003; Bakkali et al., 2008).

The essential oils exhibited the lowest MIC value (0.25 mg/mL) against *Staphylococcus aureus*, indicating a strong bactericidal effect. This is consistent with earlier studies that have reported the susceptibility of Gram-positive bacteria to essential oils (Hammer et al., 2003; Fisher & Phillips, 2008). The significant activity against *Candida albicans* (MIC of 1.0 mg/mL) and *Escherichia coli* (MIC of 0.5 mg/mL) further underscores the broad-spectrum antimicrobial potential of these oils (Cermelli et al., 2008). The variations In MIC and MBC/MFC values among different microorganisms suggest that the essential oils target multiple microbial structures and functions. The disruption of microbial cell membranes by essential oil components such as 1,8-cineole and α-pinene is a well-documented mechanism (Carson et al., 2006; Fisher & Phillips, 2008). Additionally, these compounds may inhibit critical metabolic pathways, contributing to their bacteriostatic and fungistatic effects (Juergens et al., 2003; Cushnie & Lamb, 2011). The efficacy of *Eucalyptus tereticornis* essential oils against antibiotic-resistant bacteria presents a promising alternative to conventional antibiotics, addressing the growing concern of antimicrobial resistance (Hammer et al., 2003; Stermitz et al., 2000). However, the variability in chemical composition due to environmental factors and extraction methods poses a challenge for standardization and consistent therapeutic application (Myrdal et al., 2010). Future research should focus on the detailed mechanisms of action, potential synergistic effects with other antimicrobial agents, and comprehensive clinical trials to evaluate the safety and efficacy of Eucalyptus tereticornis essential oils in therapeutic settings (Sadlon & Lamson, 2010; Lopez et al., 2011). These efforts will enhance the potential of these oils as natural antimicrobial agents in medical and industrial applications.

V. CONCLUSION

The essential oils of *Eucalyptus tereticornis* Sm. Exhibit significant antimicrobial activity against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, and fungi. The GC-MS analysis identified major constituents such as 1,8-cineole, α-pinene, and β-pinene, which are likely responsible for the observed antimicrobial effects. These findings highlight the potential of *Eucalyptus tereticornis* essential oils as natural antimicrobial agents, offering a promising alternative to synthetic antibiotics, especially in the face of rising antimicrobial resistance. However, the variability in oil composition and the need for standardization pose challenges that must be addressed. Further research, including detailed mechanistic studies and clinical trials, is essential to fully harness the therapeutic potential of these oils. This study underscores the importance of integrating traditional knowledge with modern scientific techniques to develop effective and safe natural health products.

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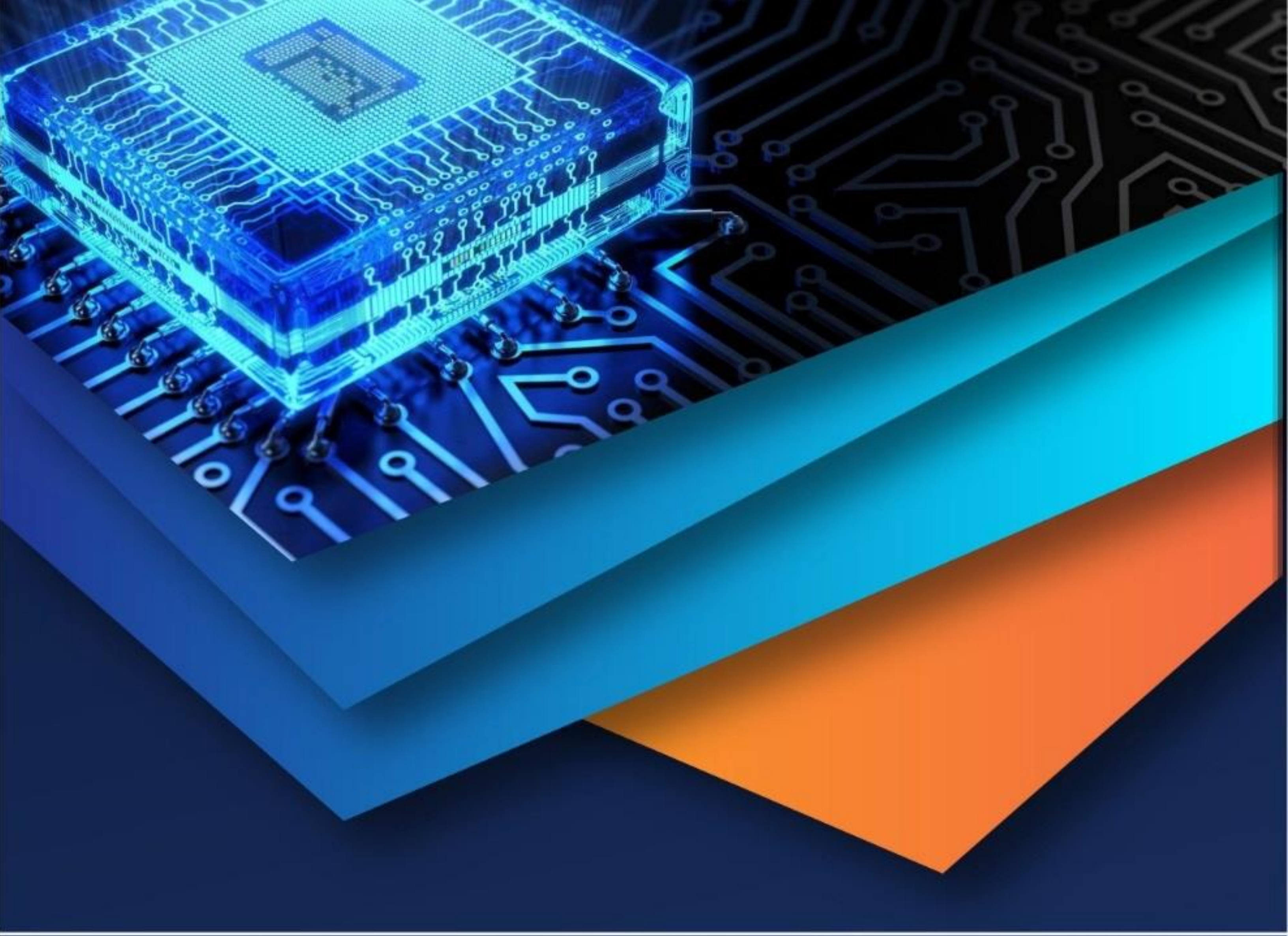

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