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Control of Indoor Aero flora using Biobased Cups

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Abstract: ***Indoor air quality is a critical concern, as poor air quality can lead to various health Issues. Microorganisms in indoor air can significantly contribute to air pollution and adversely affect human health. Therefore, effective methods for reducing microbial load in indoor environments are essential. Our study focused on isolating and identifying microbes from indoor air and evaluating a biobased fumigation cup made from natural materials for controlling indoor air microflora. Air samples were collected using the open plate technique, and microbes were isolated and identified based on their characteristics. The predominant bacteria in indoor air identified were Bacillus sp, Pseudomonas sp, Serratia sp, and Micrococcus sp and the fungi identified were Aspergillus sp Penicillium sp, Fusarium sp, and Mucor sp. The Bio-based cup is prepared using Neem, Aparajitha dhooma choornam, and Boswellia serrata resin, effectively reducing microbial load, particularly fungi, in indoor air. However, some bacterial strains showed resistance to the cup. Overall, our study highlights the potential of the biobased fumigation cup as an effective, natural method for improving indoor air quality, showing practical benefits without adverse effects, advocating for broader adoption in various applications, and encouraging environmentally conscious practices.***
Keywords: Indoor air, Neem, Aparajitha Dhooma Choornam, Boswellia serrata gum, Fumigation

I. INTRODUCTION

Indoor air quality (IAQ) is crucial for health, as poor air can cause various health issues. Microorganisms in indoor air contribute to pollution, impacting health. This project aims to isolate and analyze indoor air microbes, evaluate the effectiveness of biobased fumigation cups in reducing microbial load, and identify antimicrobial components. The study uses the open plate technique to isolate microbes, which are then cultured, analyzed morphologically, and identified using biochemical tests. Extracts from neem leaves, *Boswellia serrata* gum, and Aparajitha dhooma choornam powder will be tested for antimicrobial properties through antibiotic sensitivity tests. The impact of biobased fumigation cups on microbial load will be assessed in confined spaces, and GC-MS analysis will identify the chemical composition of natural extracts. This research will provide insights into the use of biobased fumigation cups for indoor air purification, offering sustainable, eco-friendly solutions for improving IAQ (Allen JG *et al*., 2016). IAQ is influenced by factors like humidity, temperature, and organic materials, which affect microbial load, including bacteria (e.g., *Staphylococcus*, *Streptococcus*, *Pseudomonas*), fungi (e.g., *Aspergillus*, *Penicillium*, *Cladosporium*), and viruses (e.g., influenza, rhinovirus, coronavirus) (Hospodsky *et al*., 2012; Pitkaranta et al., 2008; Prussin *et al*., 2015). Poor IAQ can cause respiratory symptoms, allergies, and infections (Adams *et al*., 2015). Biobased fumigation cups offer a sustainable alternative to chemical fumigation, using botanical extracts or microbial agents to control pests with minimal environmental impact (Isman *et al*., 2006). These cups contain active ingredients which have insecticidal, repellent, or antifungal properties, offering environmental benefits compared to chemical fumigation (Akhtar *et al*., 2004). Neem leaves (*Azadirachta indica*) are known for antimicrobial, anti-inflammatory, and antioxidant properties, widely used in organic farming and pest management (Elumalai K *et al*., 2012; Schmutterer H *et al*., 2002; Biswas K *et al*., 2022). *Boswellia serrata* gum resin, sourced from the *Boswellia serrata* tree, has anti-inflammatory, analgesic, and immunomodulatory effects, traditionally used in Ayurvedic medicine for respiratory and gastrointestinal conditions (Siddiqui, 2011). Aparajitha Dhooma Choornam powder, used in Ayurvedic "Dhoomapana," includes herbs like Vasaka, Bharangi, Ela, and Twak, known for their respiratory and antimicrobial properties (Sindhu *et al*., 2007). Biobased cups, used as an eco-friendly alternative to conventional plastic cups, reduce environmental impact by using renewable materials, being biodegradable or compostable, and supporting sustainable agriculture. However, challenges include ensuring performance, sustainable sourcing, and proper disposal to maximize environmental benefits (Narayan, 2020).

II. MATERIALS AND METHODS

1) Isolation of Microbes From Indoor Air:

Microbes were isolated from a 150 sq. ft. room in the microbiology lab at Sree Narayana Guru College, Coimbatore, using the open plate technique. Sterile Petri dishes with nutrient agar and Sabouraud Dextrose Agar (SDA) were exposed for 15-30 minutes, then incubated at 30-37°C for 24-48 hours (nutrient agar) and 48-72 hours (SDA) (Stryjakowska *et al*., 2007).

2) Identification of bacteria and fungi from indoor air:

Predominant bacteria present in indoor air were identified based on colony morphology, staining, motility and biochemical tests. Predominant fungi present in indoor air were identified based on colony Morphology and fungal Staining (Cappuccino *et al*., 2020).

3) Preparation of Biobased cup:

Biobased cups were prepared using Neem leaves, *Boswelia serrata* gum and Aparajitha dhooma choornam, an ayurvedic composition (Commercial Product) with total 8 ingredients - *Acorus calamus* , *Actiniopteris dichotoma, Aquilaria agallocha, Azadirachta indica* , *Calotropis gigantea, Cedrus deodara, Commiphora mukul* and *Shorea robusta*- used for fumigation. Fresh neem leaves were collected and thoroughly cleaned to remove any dirt or impurities. The cleaned leaves were then placed in a blender, and a small quantity of distilled water was added to facilitate proper blending and binding. Aparajitha dhooma choornam was powdered, and the powder was added to the blended neem mixture in a 1:1 ratio. This mixture was blended again to ensure uniform consistency. The homogeneous mixture was transferred to a mold and pressed to obtain the desired shape of the fumigation cup. The cups were carefully removed from the mold and placed in a shaded area to dry. They were left to dry for several days until they reached the desired texture and hardness. Finally, the cups were filled completely with 10g of *Boswellia serrata* gum. Size of the cup was 4 cm long and 2 cm wide. This biobased cup which emits fumes after burning is used for fumigation purposes.

4) Fumigation using a Biobased cup incorporated with Boswellia serrata gum resin:

Fumigation of Biobased cups is carried out by igniting till fumes start to arise. After fumes start evolving from a biobased cup for fumigation it is kept in the desired area on a ceramic stand and allowed to stand till emission of fumes is complete. *Boswellia serrata* gum resin, renowned for its antimicrobial properties, was a key component used as a fumigant in the biobased cup. This resin was incorporated into the cup, which was primarily composed of neem leaves and Aparajitha dhooma choornam powder. This combination creates a potent fumigant harnessing the natural antimicrobial properties of its ingredients. The inclusion of *Boswellia serrata* gum resin enhanced the cup's efficacy in purifying indoor air, making it a safe and natural alternative for indoor air purification.

5) Preparation of extracts from neem leaves , Boswellia serrata gum resin and Aparajitha dhooma choornam.

a) Neem leaves extract:

Fresh neem leaves were collected and thoroughly washed with sterilized water. The leaves were then shade-dried, and the dried leaves were powdered. To prepare the extract, 33.3 g of the dried powder was mixed with 100 ml of 90% ethanol and kept at room temperature for 36 hours. The slurry was filtered using Whatman No. 1 filter paper. The filtrate was evaporated and dried at 40°C, then stored in an airtight bottle at 4°C. (Subapriya *et al*., 2003)

b) Boswellia serrata gum resin extract:

Oleo-gum resin of *Boswellia serrata* was purchased from an Ayurvedic pharmacy in Alathur, Palakkad. To prepare the extract, the gum resin was powdered, and 10 g of the powder was mixed with 100 ml of 95% ethanol and kept at room temperature for 36 hours. The slurry was then filtered using Whatman No. 1 filter paper. The filtered extracts were evaporated and dried at 40°C and stored at 4°C in an airtight bottle. (Mohammadi *et al*., 2017)

c) Aparajitha dhooma choornam extract:

Aparajitha Dhooma Choornam (50g commercial packet) was purchased from an Ayurveda pharmacy in Alathur, Palakkad. For the preparation of the extract, Aparajitha Dhooma Choornam was powdered, and 16.6 g of the powder was mixed with 100 ml of 90% ethanol and kept at room temperature for 36 hours. The slurry was filtered using Whatman No. 1 filter paper. The extracts were then evaporated and dried at 40°C and stored at 4°C in an airtight bottle. (Pratap *et al*., 2019)

6) Chemical Analysis of Biobased Cup:

Chemical analysis of the components of Biobased cup were carried out using GC-MS .Separation and identification of chemical constituents of extracts from neem leaves, Aparajitha dhooma choornam, and *Boswellia serrata* gum resin were done using GC - MS analysis . (Microtech lab, Coimbatore, Tamil Nadu).

7) Determination of Antimicrobial activity of Neem, Aparajitha dhooma choornam, and Boswellia serrata gum resin extracts:
Antimicrobial properties were analyzed using the well diffusion method. (Khan *et al.*, 2012). Using a sterile swab, broth cultures of the inoculum was swabbed on to Mueller-Hinton agar (MHA) media and 0.1ml Extracts of neem, Boswellia serrata gum, and Aparajitha dhooma choornam were added to bored wells on the agar. The plates were then incubated at 37°C for 24 hours for bacteria and at room temperature for 24 -72 hours for fungi. The zones of growth inhibition were recorded against the test microorganisms.

8) Indoor air fumigation using Biobased Cup:
One biobased cup for fumigation containing neem leaves, Aparajitha dhooma choornam, and *Boswellia serrata* gum resin was used to fumigate a room of 150 square feet at Sree Narayana Guru College. Effectiveness was assessed by comparing microbial load before and after fumigation by open plate technique using sterile nutrient agar plates and Sabouraud Dextrose Agar plates (Prasannakumar and Balachandran, 2021).

9) Effectiveness of fumigation using a Biobased cup for controlling indoor air microflora:
Effectiveness was evaluated by comparing the microbial load on agar plates exposed before and after fumigation of indoor air. Open plate technique was carried out before 15 minutes of fumigation and after 15 minutes of fumigation. After incubating nutrient agar plates at 30-37°C for 24 hours and Sabouraud's Dextrose Agar plates at room temperature for 24-72 hours the colonies grown in all the plates were tabulated , identified, compared and assessed.

III. RESULT

1) *Isolation of Microbes from Indoor Air:* Using the open plate technique, bacterial and fungal colonies were isolated (Fig. 1 and Fig. 2). Four bacterial and four fungal colonies with distinct morphologies were selected for identification.

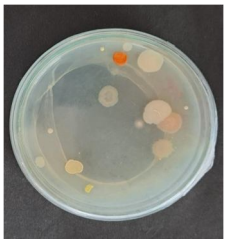


Fig 1: Bacterial colonies on nutrient agar plates

Fig 2:- Fungal colonies on SDA.

2) *Identification of Microorganisms from Indoor air:* Bacterial and fungal isolates were identified based on colony morphology, staining, motility, and biochemical tests. The predominant bacteria in indoor air were identified as *Bacillus, Pseudomonas, Serratia, and Micrococcus, and fungi were identified as Aspergillus, Penicillium, Fusarium, and Mucor. (Fig 3-16)*

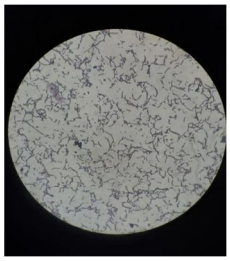


Fig 3: Gram-positive rod.

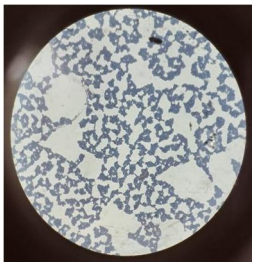


Fig 4: Gram-positive cocci.

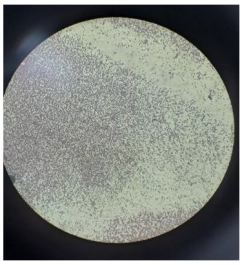


Fig 5: Gram-negative rod


Fig 6: Indole test.


Fig 7: Methyl red test.


Fig 8: Voges-Proskauer test


Fig 9: Citrate test.


Fig 10: Nitrate test.


Fig 11:Urease test


Fig 12:TSI test

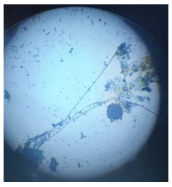


Fig 13:- Aspergillus

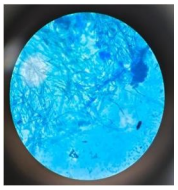


Fig 14:- Penicillium

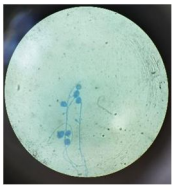


Fig 15:- Mucor

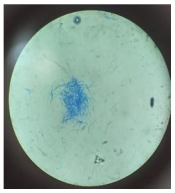


Fig 16:- Fusarium

3) *Preparation of Biobased Cup:* Fresh neem leaves were collected and thoroughly cleaned to remove any dirt or impurities. These cleaned leaves were then placed in a blender, and a small quantity of water was added to facilitate proper blending and binding. Aparajitha Dhooma Choornam was powdered, and the powder was added to the blended neem mixture. The mixture was blended again thoroughly to ensure a homogeneous consistency. The mixture was then transferred into a mold and pressed to obtain the desired shape of the fumigation cup. The cups were carefully separated from the mold and placed in a shaded area to dry. They were left to dry for several days until they achieved the desired texture and hardness. The cups were then incorporated with *Boswellia serrata* gum and prepared for fumigation.


Fig 17:- Biobased cups for fumigation

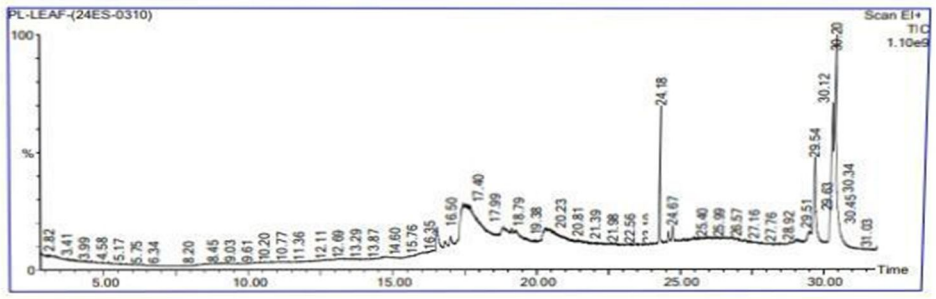
4) *Preparation of Extract:* Ethanolic extracts of Neem, Aparajitha dhooma choornam, and *Boswellia serrata* gum resin were prepared. The Neem extract was dark green and syrupy, the Aparajitha dhooma choornam extract was dark brown and watery, and the *Boswellia serrata* extract was yellowish-brown and sticky.


Fig 18: Neem extract.


Fig 19: Aparajitha dhooma choornam extract


Fig 20: *Bosweilla serrata* Gum resin extract.

5) *GC-MS Analysis:* By GC-MS analysis most abundant 10 major compounds present in the mixture of biobased cup with neem, *Boswelia serrata* gum and Aparajitha dhooma choornam containing 8 different herbs is 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-cyclohexanol as the most abundant, known for its strong antimicrobial properties.



#	RT	Scan	Height	Area	Area %	Norm %
1	16.509	2741	112,182,608	10,827,937.0	2.561	7.83
2	17.519	2943	192,918,464	138,340,752.0	32.715	100.00
3	18.790	3197	75,123,080	29,440,752.0	6.962	21.28
4	19.095	3258	67,446,344	6,261,747.0	1.481	4.53
5	19.220	3283	60,700,720	16,860,782.0	3.987	12.19
6	20.260	3491	67,078,668	37,442,340.0	8.854	27.07
7	24.182	4275	639,866,240	20,455,818.0	4.837	14.79
8	29.554	5349	366,731,136	29,682,080.0	7.019	21.46
9	30.124	5463	649,768,832	37,435,048.0	8.853	27.06
10	30.199	5478	961,862,144	96,116,568.0	22.730	69.48

Sl.No	RT	Name of compound	Properties
1	16.509	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL (phytol)	• Solubility: It is insoluble in water but soluble in organic solvents. • It is stable under normal conditions but may undergo oxidation over time. • Activity: It has antioxidant, anti-inflammatory, and anticancer properties.
2	17.519	2-O-METHYL-D-MANNOPYRANOSA(D-mannose)	•Solubility: It is soluble in water. •Stability: It is stable under normal conditions. •Prevent urinary tract infections by inhibiting microbe's adhesion to the urinary tract lining.
3	18.790	U*NDECANOIC* ACID (undecylic acid)	•Solubility: It is slightly soluble in water but more soluble in organic solvents. • Stability: Stable under normal conditions. •Biological properties: Antifungal and Antibacterial properties.
4	19.095	BETA.-D-MANNOFURANOSIDE, METHYL (Methyl)	•Solubility: It is soluble in water. •Stability: It is stable under normal conditions. • Lacks antimicrobial properties but can enhance antimicrobial activity when part of a compound.
5	19.220	ETHER, HEXYL ISOPROPYL	•Solubility: It is soluble in organic solvents but not very soluble in water. •Odor: It has a mild, ether-like odor. •Flammability: It is flammable
6	20.260	3-DECYN-2-OL	•Odor: It has a characteristic odor. •Solubility: It is slightly soluble in water but soluble in organic solvents.

			•Biological properties: Antifungal And Antibacterial properties
7	24.182	SQUALENE	•Solubility: Insoluble in water, soluble in organic solvents. •Medical Use: Investigated for its potential health benefits, including antioxidant and anti-inflammatory properties. •Generally considered safe and non-toxic for topical and oral use.
8	29.554	OCTAMETHYL	•Solubility: Insoluble in water, soluble in organic solvents like benzene, ether, and acetone •Reactivity: Generally stable under normal conditions, • Odor: Odorless
9	30.124	2R-ACETOXYMETHYL-1,3,3-TRIMETHYL-4T-(3-METHYL-2-BUTEN-1-YL)-1T-CYCLOHEXANOL	•It has antimicrobial properties •It has antioxidant activities •It has larvicidal activities
10	30.199	5H-3,5A-EPOXYNAPHTH[2,1-C]OXEPIN, DODECAHYDRO-3,8,8,11A-TETRAMETHYL	•It exists as a colorless to pale yellow liquid. • It has Antibacterial activities •It has antifungal activities

Table 4: Compounds present in mixture of Biobased cup

6) *Determination of Antimicrobial Properties:* The antimicrobial effect of Neem, Aparajitha dhooma choornam, and *Boswellia serrata* gum extracts were tested on bacteria (*Serratia, Staphylococcus, Bacillus, Micrococcus*) and fungi (*Aspergillus, Penicillium, Mucor, Fusarium*) using the well diffusion technique. All microorganisms showed inhibition zones, indicating they could not grow in the presence of these extracts (Fig.18a - Fig.18e).

Extracts	Zone of inhibition				
	Bacillus sp	*Serratia sp*	*Staphylococcus sp*	*Bacillus sp*	*Micrococcus sp*
Neem	21mm	35mm	31mm	24mm	13mm
Aparajitha dhooma choornam	16mm	21mm	25mm	17mm	12mm
Bosweilla seratta gum	13mm	14mm	16mm	17mm	12mm

Table 5: Antimicrobial properties of herbal extracts against isolated bacteria.

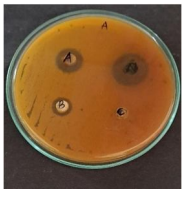


Fig 21:- *Bacillus sp.*

Fig 22:- *Serratia sp.*

Fig 23:- *Staphylococcus sp*

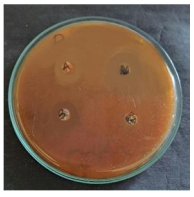


Fig 24:- *Bacillus sp.*

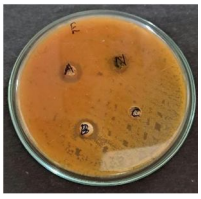


Fig 25:- *Micrococcus sp.* `

Extracts	Zone of inhibition			
	Aspergillus	*Penicillium*	*Mucor*	*Fusarium*
Neem	20mm	23mm	21mm	12mm
Aparajitha Dhooma Choornam	17mm	20mm	20mm	13mm
Bosweilla seratta gum	14mm	15mm	19mm	14mm

Table 6: Antimicrobial properties of herbal extracts against isolated fungi.

Fig 26:- *Aspergillus sp.*

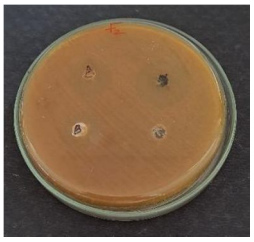


Fig 27:- *Penicillium sp.*

Fig 28:- *Mucor sp.*

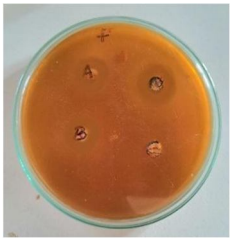


Fig 29:- *Fusarium*

7) *Fumigation using Biobased Cup***:** The cup was dried and then incorporated with *Boswellia serrata* gum. It was fumigated in a single room from the UG lab of Sree Narayana Guru College, Chavadi, Coimbatore, for 20 minutes, where the microbes had been previously isolated, to assess the reduction in microbial load using the open plate technique. After fumigation, Sabouraud Dextrose Agar (SDA) and Nutrient Agar plates were exposed using the open plate technique to observe the reduction in microbial load.

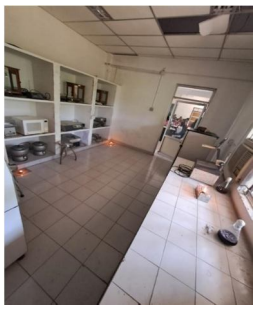


Fig 30: Fumigation area

Fig 31: Fumigation using a Biobased cup

8) *Effectiveness of Fumigation:* After fumigation there was a noticeable reduction in microbial load in indoor air.The biobased cup was more effective against fungi than bacteria. Although *Bacillus sp.* and *Micrococcus sp*. showed resistance, their numbers were reduced. *Aspergillus sp*. also exhibited resistance, while other fungi were sensitive. Fumigation was particularly more effective in controlling mold than bacteria.

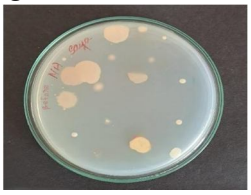


Fig 32: Bacterial colonies on nutrient agar plate before fumigation

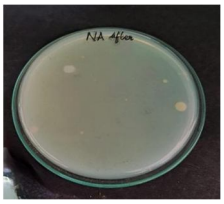


Fig 33: Bacterial colonies on nutrient agar plate after fumigation

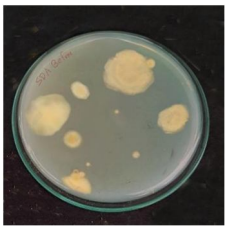


Fig 34: Fungal colonies on the SDA plate before fumigation

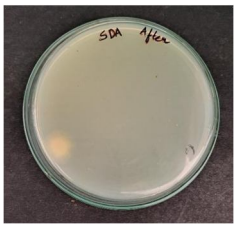


Fig 35: Fungal colonies on SDA plate after fumigation

IV. DISCUSSION

The Indoor air microflora was isolated using the open plate technique and identified through Gram staining and biochemical tests, revealing five predominant bacterial cultures: *Bacillus*, *Serratia*, *Staphylococcus*, and *Micrococcus*. Fungal colonies were identified using lactophenol cotton blue staining, resulting in Aspergillus, Penicillium, Mucor, and Fusarium. The biobased cup (Neem, Aparajitha dhooma choornam, *Boswellia serrata* gum resin) exhibited a greenish-brown color and slightly rough texture, indicating organic materials. The extracts showed significant antimicrobial activity against the isolated bacteria and fungi. Fumigation with the cup led to a noticeable reduction in microbial presence, especially fungi. After fumigation, *Bacillus* and *Micrococcus* were identified, suggesting resistance to the fumigant, while *Penicillium* was the predominant fungus, indicating resilience to the fumigant. These findings highlight the potential of biobased fumigation cups for indoor air purification (public health, environmental sustainability). Future research could optimize the fumigation process and explore additional natural extracts.

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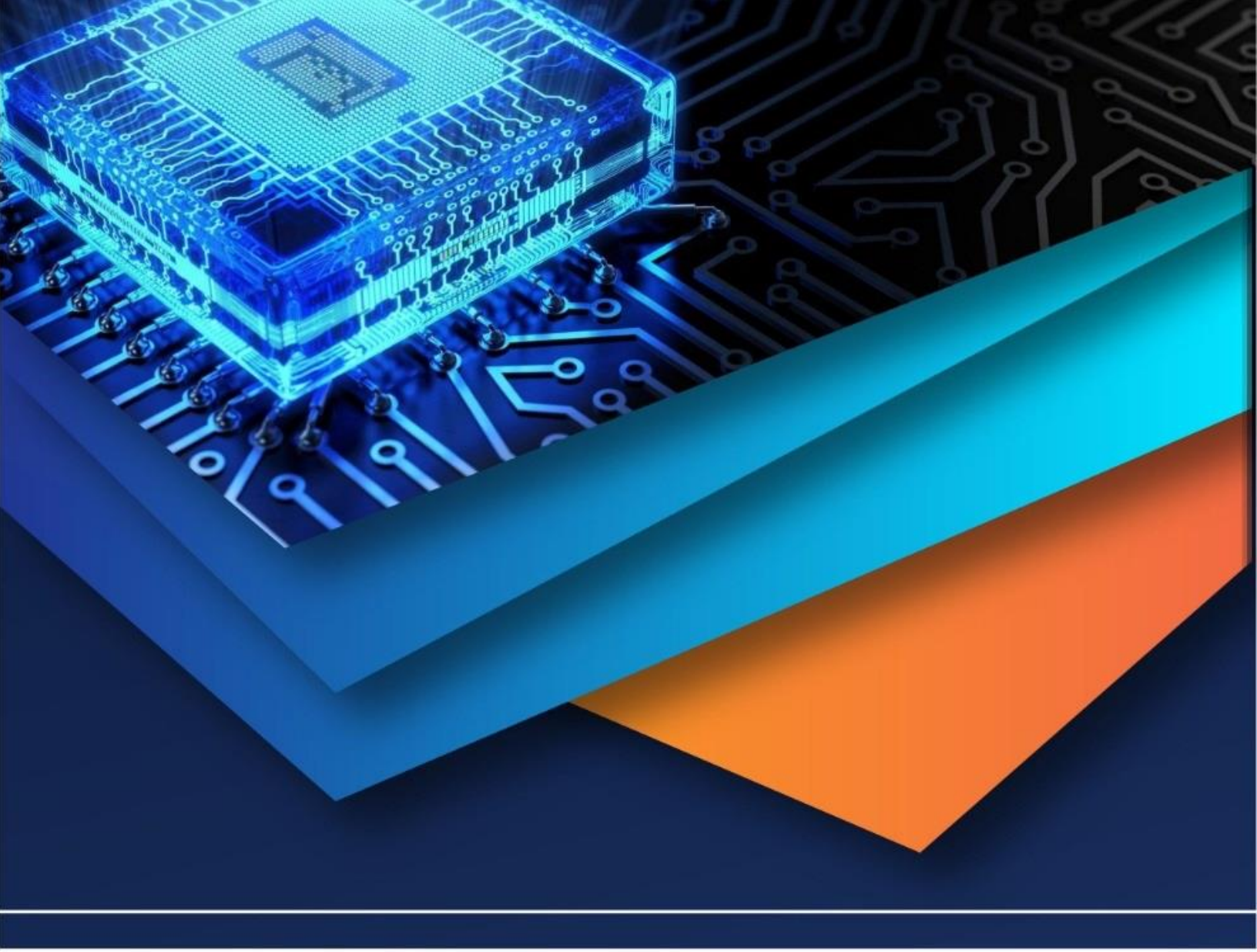

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