iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** IX **Month of publication:** September 2017

DOI: http://doi.org/10.22214/ijraset.2017.9188

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

A Study on the Extraction Process of Wrightia Tinctoria and Evaluation of Its Antimicrobial Activity

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Abstract: ***Response Surface Methodology (RSM) with Central Composite Design (CCD) is performed in the present study under different experiment runs varying the weight of the Wrightia tinctoria leaf powder. Aqueous, ethanol and ethyl acetate extracts are derived from Wrightia tinctoria plant leaf powder using Soxhlet apparatus under various experimental conditions. Antimicrobial activity of the plant extracts are investigated using agar disc diffusion method.***
Keywords: Response Surface Methodology, Central Composite Design, Experiment Runs, Wrightia tinctoria, Soxhlet Apparatus

I. INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants is grown in different parts of the country. Ayurveda, Unani and Siddha are systematically used nearly 1500 plants in indigenous system of medicine. Medicinal plants are the oldest existing complete medical system in the world. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases (Saleh et.al., 2009).

Uses of medicinal plants in the industrialized societies have been traced from the extraction and development of several drugs and chemotherapeutic drugs from these plants as well as from traditionally used rural herbal remedies (Sharma et.al., 2010). Wrightia tinctoria (Syn. Pala indigo plant) of Apocyanaceae family is widely used in skin diseases, liver disorders and broad spectrum biological activities (Kirtikar and Basu, 1981).

The plant is used to treat seizures, wounds, leukemia, gynecological disorders, toothache, headache, dandruff, diarrhea and skin disorders like psoriasis, eczema, scabies etc. *Wrightia tinctoria* has found its way into unani formulation called Hab-e-Jund which is being used to treat febrile convulsions and also is being proposed for the treatment of generalized tonic-clonic seizure and absence seizure (Koneru et.al., 2009). The whole plant or its specific parts (bark, leaf, seed and root) are known to have medicinal properties and have a long history of use by indigenous communities in India (Nadkami, 1976). A range of techniques, varying in cost and level of complexity, may be used for extraction of plant material. Methods of Soxhlet extraction can be classified as continuous or discontinuous.

In continuous methods (e.g., percolation and soxhlet extraction), solvent flows through the plant material continuously. In the case of discontinuous methods, the solvent is added and removed in batches (Sarkar et.al., 2006). The statistical approach to experimental design is necessary if we wish to draw meaningful conclusions from the data. When the problem involves data that are subject to experimental errors, statistical methods are the only objective approach to analysis (Montgomery, 2017). DOE refers to the process of planning, designing and analysing the experiment so that valid and objective conclusions can be drawn effectively and efficiently. In order to draw statistically sound conclusions from the experiment, it is necessary to integrate simple and powerful statistical methods into the experimental design methodology (Antony, 2014).

In the present study, Design of Experiments (DOE) is employed using the Design Expert- Stat Ease software for improving the quality of the extract.

This software is user-friendly, helping in screening, characterization, optimization and validation. It helps in modifying the complex processes into simpler designs exploring the graphs in 2D and 3D forms at all angles. Optimization work is usually carried out using this statistical software thus saving time and cost of the further processing. Under various types of experimental designs, Central Composite Design (CCD) which is the most popular response surface design is selected for the present study. The software calculates the number of runs to carry out the extraction process. The main aim of the study is to calculate the time taken for extraction, number of cycles taken, final weight and yield of the extract in different runs varying the weight of the plant powder and to study the antimicrobial activity of the viscose non woven fabric loaded with the prepared extracts against Escherichia Coli and Methicillin Resistant Staphylococcus aureus.

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II. MATERIALS AND METHODS

A. Plants and Chemicals

Wrightia tinctoria leaves are used to make the plant extract. The plant is selected for the following reasons; Cost effectiveness, Ease and Availability, Medicinal Properties, Less toxic, Energy efficient and Ecofriendly nature.

B. Taxonomic Classification

Botanical name: *Wrightia tinctoria* R.Br.
Family - Apocynaceae
Kingdom - Plantae
Genus -Wrightia
Species - tinctoria

The solvents used for extraction are water, ethanol and ethyl acetate. Double distilled water is used for the experiments. All the chemicals were purchased from Precision scientific chemicals, Coimbatore and are of analytical grade and are used without further purification.

C. Microorganisms and Growth Conditions

The test strains, Gram negative bacteria Escherichia Coli and Gram positive bacteria Methicillin Resistant Staphylococcus aureus are obtained from the Department of Microbiology, Periyar University, Salem. These bacterial strains are cultured and these representative microorganisms are used to evaluate the antimicrobial activity of the extract.

D. Identification of Plant Sample

During the period of flowering and vegetative phase, sample of *Wrightia tinctoria* parts consisting of leaves, stem, flower buds are collected for getting authentication. By comparing the collected specimen with that of a known identity available in the herbarium of Botanical Survey of India, TNAU Campus, Coimbatore, the taxonomic identity of the plant is confirmed.

E. Collection of Plant Sample

Fresh and healthy leaves are collected from the agricultural field near Erode in the month of February.

Fig. 1. Collected leaves

F. Preparation of Leaf Powder

The collected leaves are washed thoroughly twice with running tap water followed by double distilled water to remove all debris and other contaminated organic contents. They are kept away from direct sunlight to avoid destruction of active compounds. They are shade dried for 20 days to remove the water from the surface of the leaves and then ground into a fine powder using domestic blender. The powder is refrigerated in an airtight container for further use.

G. Extraction Method

Soxhlet extractor is used for extracting the plant material. The dry leaf powder sample is extracted with water, ethanol and ethyl acetate solvents using Soxhlet extractor until complete extraction.

Fig. 2.Soxhlet extractor

H. Extraction Procedure

The *Wrightia tinctoria* leaf powder to be extracted in taken in the required quantity as per the experimental runs and loaded in a thimble made from filter paper. Then the thimble is placed inside the extractor and the required solvent in 200 ml is taken in the conical flask which is placed about the heating element. When the apparatus is turned on, the vapour from the solvent travels through the distillation arm and then through the thimble. The thimble is surrounded by the warm solvent which makes the powder to get dissolved in it. When the solvent is full in that area, then it gets automatically emptied to the conical flask placed at the bottom. The cycle is repeated until the solvent becomes colourless. The extracted solvent is taken and placed in a rotary evaporator. Here the solvent is evaporated and the dry crude extract is transferred to the vial for the next activity.

Fig. 3. Prepared extracts

I. Experimental Runs- Design Expert Software

Design Expert software is used for calculating the number of runs for extraction. Design–Expert is a statistical software package from Stat-Ease Inc. that is specifically dedicated to performing design of experiments (DOE). Central composite design is selected under response surface model. Response surface methodology(RSM) of single factor i.e. Concentration of plant powder is taken for the study and employed against four responses and the experiment is carried out in 7 runs. Effect of the concentration of plant powder against the responses (Time taken for extraction, final weight of the solvent, number of cycles and yield (%)) are recorded along with antimicrobial activity.

J. Yield of the Extract

The yield of the extracts is calculated by using the formula

$$\text{Total extract yield (\%)} = \frac{\text{Total mass of extract}}{\text{Total mass of sample}} \times 100$$

K. Antimicrobial Activity

The plant extracts are taken and 20 µl of the extract is loaded on the viscose nonwoven fabric samples for evaluating the antimicrobial activity against Gram negative bacteria Escherichia Coli and Gram positive bacteria Methicillin Resistant Staphylococcus aureus. These bacterial strains are cultured and these representative microorganisms are used to evaluate the antimicrobial activity of the extract and the loaded fabric sample using agar disc diffusion method. The diameter of zone of inhibition found on the agar plates observed in the antimicrobial study is recorded.

L. Nomenclature of the Samples

Table 1: Nomenclature of The Samples And Experimental Runs

S.No.	SAMPLE NAME			RUN ORDER	WEIGHT (IN GRAMS)
	SOLVENT USED FOR EXTRACTION				
	AQUOEUS	ETHANOL	ETHYL ACETATE		
1	A1	EL1	EA1	1	2
2	A2	EL2	EA2	2	6
3	A3	EL3	EA3	3	3
4	A4	EL4	EA4	4	4
5	A5	EL5	EA5	5	6
6	A6	EL6	EA6	6	2
7	A7	EL7	EA7	7	5

III. RESULTS AND DISCUSSION

A. Preparation of Plant Extract- Time Taken

Table 2 Preparation Of Plant Extract- Time Taken

S.No.	Samples	Run Order	Concentration of plant powder (grams)	Time taken for extraction (minutes)
1	A1	1	2	107
	EL1			85
	EA1			49
2	A2	2	6	212
	EL2			181
	EA2			82
3	A3	3	3	157
	EL3			117
	EA3			61
4	A4	4	4	154
	EL4			209
	EA4			80
5	A5	5	6	160
	EL5			160
	EA5			65
6	A6	6	2	84
	EL6			116
	EA6			63
7	A7	7	5	159
	EL7			149
	EA7			87

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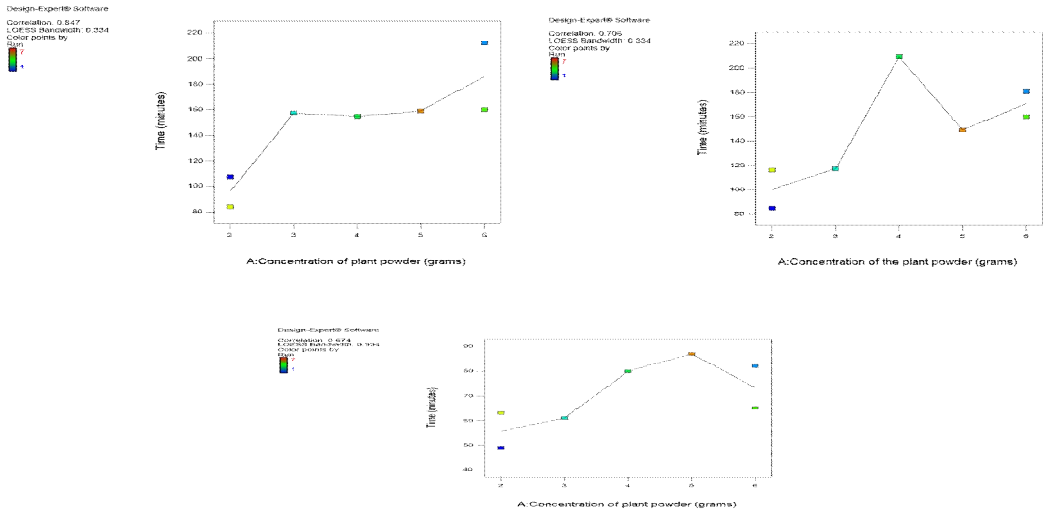

Fig 4. Concentration of plant powder Vs Time – Aqueous, Ethanol and Ethyl acetate extracts- Design Expert Software

It is clear from the table that majority of samples in 6g concentrations has taken maximum time for extraction whereas majority of samples in 2g concentrations has taken minimum time for extraction. It is also proved from the runs that time increases as the weight of the plant powder increase. It is found that A2 sample (6g concentration), EL4 sample(4g concentration) and EA7 sample (5g concentration)has taken a maximum time of 212 minutes, 209 minutes and 87 minutes respectively among Aqueous, ethanol and ethyl acetate solvents used for extraction. The graphs from the software also proved there is a positive correlation of 0.847, 0.706 and 0.674 found between concentration of plant powder and time taken for extraction using aqueous, ethanol and ethyl acetate solvents respectively.

B. Preparation of Plant Extract- Number of Cycles

TABLE 3 PREPARATION OF PLANT EXTRACT- NUMBER OF CYCLES

S.No.	Samples	Run Order	Concentration of plant powder (grams)	No. of cycles
1	A1	1	2	7
	EL1			12
	EA1			3
2	A2	2	6	7
	EL2			11
	EA2			7
3	A3	3	3	5
	EL3			16
	EA3			7
4	A4	4	4	6
	EL4			9
	EA4			9
5	A5	5	6	8
	EL5			6
	EA5			9
6	A6	6	2	7
	EL6			5
	EA6			3
7	A7	7	5	4
	EL7			10
	EA7			10

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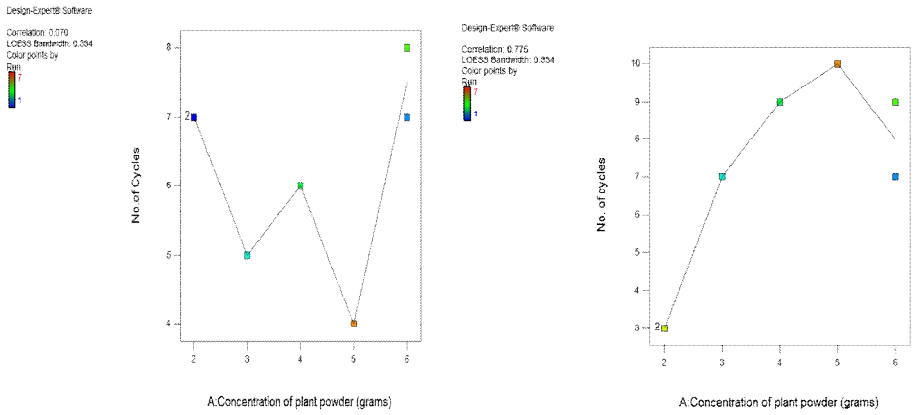

Fig. 5. Concentration of plant powder Vs Number of cycles – Aqueous and Ethyl acetate extracts- Design Expert Software

The results showed that the weight of the plant powder didn't impart any influence on the number of cycles in extraction using aqueous and ethanol solvents. It is found that the extraction using ethanol has taken maximum number of cycles compared to the others. There is a positive correlation of 0.070 and 0.776 found between concentration of plant powder and number of cycles for extraction using aqueous and ethyl acetate solvents.

C. Preparation of Plant Extract- Final Weight of the Extract

TABLE 4 PREPARATION OF PLANT EXTRACT- FINAL WEIGHT OF THE EXTRACT

S.No.	Samples	Run Order	Concentration of plant powder (grams)	Initial weight (ml)	Final weight (ml)
1	A1	1	2	200	155
	EL1				185
	EA1				180
2	A2	2	6		136
	EL2				180
	EA2				160
3	A3	3	3		160
	EL3				182
	EA3				170
4	A4	4	4		175
	EL4				185
	EA4				170
5	A5	5	6		163
	EL5				170
	EA5				176
6	A6	6	2		185
	EL6				179
	EA6				170
7	A7	7	5		162
	EL7				178
	EA7				168

It is found that A2, EL5, EA2 samples (6g concentration) has reduced weight of 136ml, 170ml and 160ml compared to other samples respectively. It is clear that majority of the ethyl acetate samples (EA3, EA4 and EA6) showed 170ml after extraction. It is also found from the table that aqueous extraction showed reduced weight compared to the others.

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D. Preparation of Plant Extract- Yield of the Extract

TABLE 5 PREPARATION OF PLANT EXTRACT- YIELD OF THE EXTRACT

S.No.	Samples	Run Order	Concentration of plant powder (grams)	Yield (%)
1	A1	1	2	77.5
	EL1			92
	EA1			90
2	A2	2	6	68
	EL2			90
	EA2			80
3	A3	3	3	80
	EL3			91
	EA3			85
4	A4	4	4	87.5
	EL4			92
	EA4			85
5	A5	5	6	81.5
	EL5			85
	EA5			88
6	A6	6	2	92.5
	EL6			89.5
	EA6			85
7	A7	7	5	81
	EL7			89
	EA7			84

It is found that the samples extracted using ethanol showed good yield compared to the others. It is found that A6 sample (2g concentration), EL1 (2g concentration) & EL4 (4g concentration) samples and EA1 sample (2g concentration) has showed maximum yield of 92.5 %, 92% and 90% respectively.

E. Preparation of Aqueous Plant Extract-One Factor Graph and Predicted Vs Actual Graph

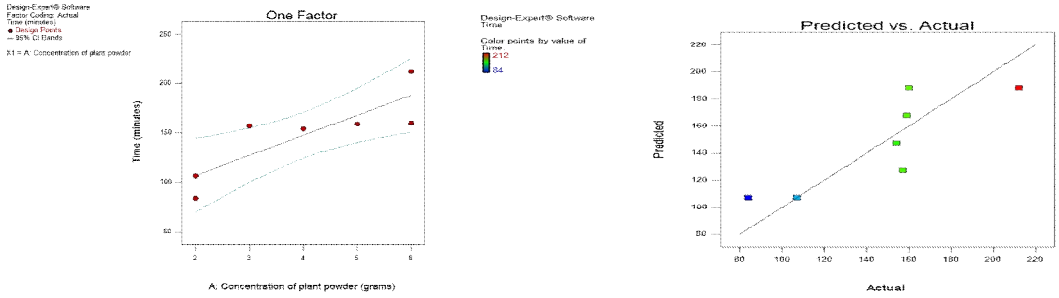

Fig. 6. Aqueous

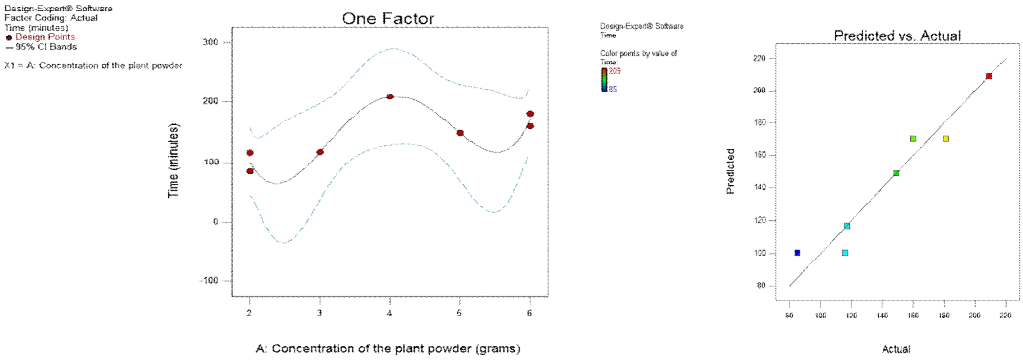

Fig. 7. Ethanol

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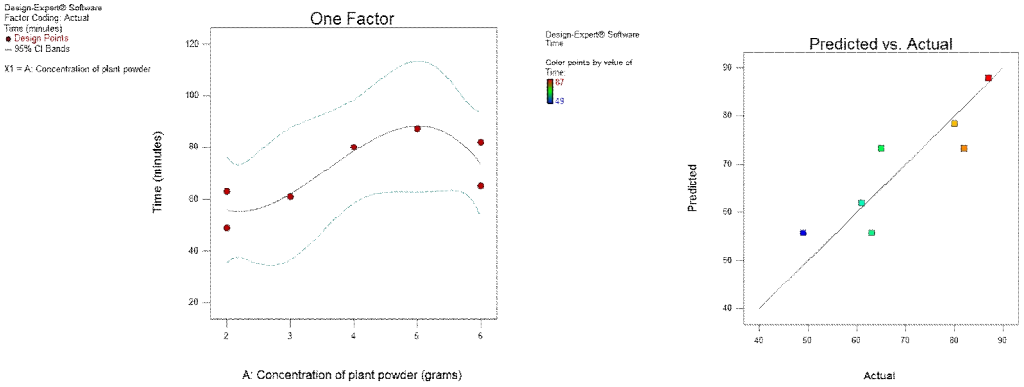

Fig. 8. Ethyl acetate

F. Antimicrobial Activity

TABLE 6 AQUEOUS EXTRACTS

SAMPLES	WEIGHT (g)	E.COLI				MRSA			
		PLANT EXTRACT		LOADED FABRIC		PLANT EXTRACT		LOADED FABRIC	
		24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS
A1	2	8	10	0	0	0	0	0	0
A2	6	0	0	0	0	0	0	0	0
A3	3	0	0	0	0	0	0	0	0
A4	4	0	0	0	0	0	0	0	0
A5	6	0	0	0	0	0	0	0	0
A6	2	0	0	0	0	0	0	0	0
A7	5	11	11	0	0	0	0	0	0

The antimicrobial activity against *E-coli* was found only in A1(2g concentration) and A7 (5g concentration) plant extracts with 10mm and 11mm (after 48 hours) zone of inhibition respectively. It is clear that no other samples showed antimicrobial activity against both the bacteria in both plant extracts and loaded fabrics.

TABLE 7 ETHANOLIC EXTRACTS

SAMPLES	WEIGHT (g)	E.COLI				MRSA			
		PLANT EXTRACT		LOADED FABRIC		PLANT EXTRACT		LOADED FABRIC	
		24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS
EL1	2	0	0	0	1	0	0	0	1
EL2	6	0	5	7	18	0	9	7	22
EL3	3	0	2	0	6	0	1	0	5
EL4	4	0	3	0	8	0	1	0	7
EL5	6	0	6	9	17	0	11	9	25
EL6	2	0	0	0	3	0	0	0	3
EL7	5	0	4	0	6	0	4	0	6

It is found that EL2 and EL5 samples (6g concentration) exhibit larger diameter of zone of inhibition in both the plant extracts and loaded fabrics against both the bacterial strains.

Table 8 ETHYL Acetate Extracts

SAMPLES	WEIGHT (g)	E.COLI				MRSA			
		PLANT EXTRACT		LOADED FABRIC		PLANT EXTRACT		LOADED FABRIC	
		24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS	24 HRS	48 HRS
EA1	2	0	0	0	0	0	0	0	0
EA2	6	0	1	3	7	0	5	7	18
EA3	3	0	0	0	6	0	0	0	4
EA4	4	0	0	0	7	0	0	0	6
EA5	6	0	3	5	9	0	5	6	14
EA6	2	0	0	0	3	0	0	0	0
EA7	5	0	0	0	6	0	2	0	3

It is found that EA2 and EA5 samples (6g concentration) exhibit larger diameter of zone of inhibition in both the plant extracts and loaded fabrics against both the bacterial strains.

IV. CONCLUSION

The results of the present investigation showed the detailed procedure for the extraction of Wrightia tinctoria leaf extract, a plant that shelters greater medicinal potential along with the experimental runs carried out for extraction. The study explored the results with respect to the variation in the weight of the plant powder. It is found that the ethanolic leaf extracts of Wrightia tinctoria have good antimicrobial activity against E.Coli and MRSA followed by ethyl acetate and aqueous leaf extracts. The results are found to be good in all the aspects in the higher concentration of the plant powder (6g).

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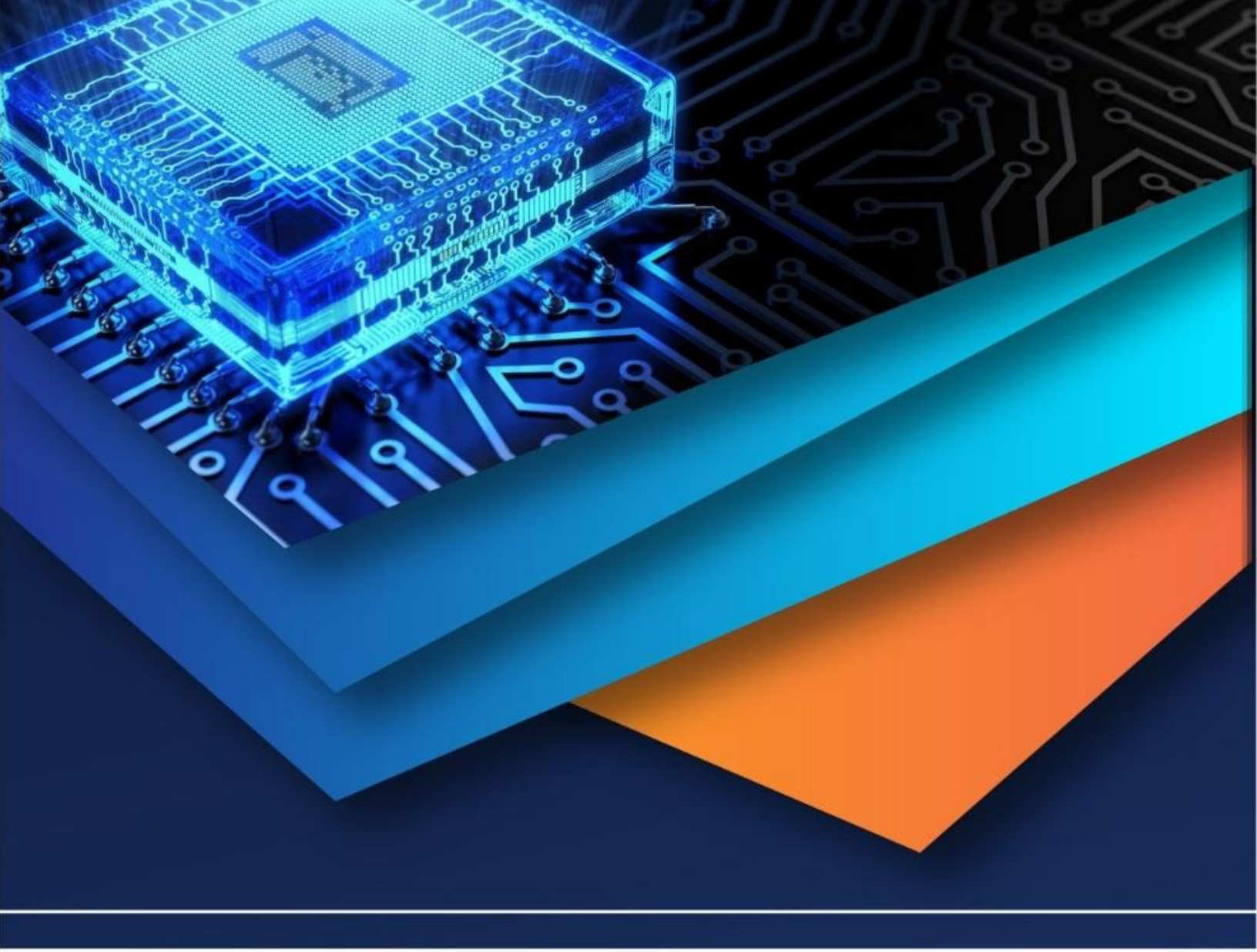

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