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Formulation and Evaluation of Giloy Berberine-Loaded Hydrocolloidal Patches for Acne Treatment

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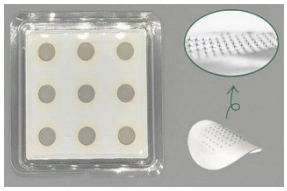
Abstract: ***Giloy, scientifically known as Tinospora Cordifolia, is a widely recognized medicinal plant in Ayurveda. It is a deciduous climbing shrub with heart-shaped leaves belonging to Menispermaceae family, acting as one of the main sources of new pharmaceuticals and health care products. The plant is genetically diverse, containing different active components, including steroids, aliphatics, alkaloids, glycosides, and diterpenoid lactones. This herb possesses anti- inflammatory, antibacterial, immunomodulating agent, anti-viral, anti-diabetic properties in addition to being a strong antioxidant.[1] A primary method for determining the antimicrobial activity of a compound and its active substance was carried out on invitro model through agar well diffusion method on Propionobacterium Acne. Observations of antibacterial activity were carried out by reviewing clear zones indicating the inhibition zones.[2] Studies revealed that T. cordifolia is an excellent drug and does not have any adverse or toxic effects till now. There is a new invention for topical acne treatment called acne patch. The purpose of this study was to formulate an acne patch preparation from Giloy Stem extract with a combination of Xanthan gum and Propylene glycol and other excipients against Propionibacterium acnes bacteria. Acne patch preparation was evaluated for physical characteristics, and tested for its antibacterial effectiveness activity.***

Keywords: Giloy, P. acne, Hydrocolloidal Patches, T.Cordifolia, Berberine, Xantham Gum.

I. INTRODUCTION

Patches as a topical acne treatment:

Acne is commonly affecting many teenagers that makes so many scientists are exploring many formulas for acne treatment. There is a new invention for topical acne treatment called acne patch. In general, acne patches that are already on the market contain hydrocolloid synthesizing substances.[3]Acne patches on the market are usually made of hydrocolloids or hydrogels. Hydrocolloid dressings consist of two layers, namely, a colloid layer and a water impermeable layer. The so-called colloid layer is the inner layer and the water-impermeable layer is the outer layer. The function of the water-impermeable layer is that it can provide a protective layer and can help prevent the spread of pathogenic microorganisms, Based on the type of acne, acne patches are divided into several types:

Sr.no.	Acne Patches	Characteristics	
1	Microneedle patches	• For cystic or nodular acne Containing microneedle that is dissolved and is very fine, has a small needle on one side Can help deliver and penetrate the active ingredients into the deeper layers of the skin	 Fig. 1 Microneedle patch
2	Hydrogel patches	For active acne contains active ingredients to kill acne-causing bacteria and reduce inflammation. Can reduce bumps, pain, and redness in pimples, and can relieve inflamed pimples such as papules. Can also help reduce the size of lesions due to nodular or cystic acne.	 Fig. 2 Hydrogel Patch

3	Hydrocolloid patches	For yellow or white pimples. Often used to speed up the healing process. Has the size of a pimple and is circular in shape. The shape is also very thin so that when used in public it is less visible. Can suck moisture from the pores, prevent other infections, and can prevent the formation of acne scars on the skin.	 Fig. 3 Hydrocolloid Patch

A. Hydrocolloidal Patches:

Hydrocolloid acne patches primarily target the Epidermis , specifically its outermost layers.

Targeted Skin Layer:

Stratum Corneum*(the outermost layer of the epidermis)*

- This is where the patch adheres and forms a moist environment.
- It absorbs fluid/ pus from whiteheads or popped pimples.
- Helps isolate the wound from bacteria, dirt, and friction.
- Hydrocolloid patches are is non medicated acne patch used to treat acne.
- The function of this patch is to give skin hydration and improve the skin barrier.

The Permeation mechanism of Hydrocolloid patches is passive and absorptive, targeting superficial acne lesions like whiteheads and pustules.

Particularly effective for surface-level acne (not deep cystic acne).

Can flatten pimples overnight by drawing out pus and reducing inflammation.

No chemical interaction with deeper layers of the skin.

II. TINOSPORA CORDIFOLIA (GILOY)

Classification of Plant:

Biological Source: Giloy is a deciduous climbing shrub that is tropical to Indian subcontinents.

Species: Cordifolia

Family: *Menispermaceae*

Kingdom: Plantae

Genus: Tinospora

Synonyms: Gulvel, Guduchi, Giloy

Fig. 4 T.Cordifolia

III. CHEMICAL CONSTITUENTS

Various chemical constituents have been found in different parts of the Giloy plant. They belongs to different classes such as alkaloids, terpenoids, steroids, glycosides, polysaccharides, flavonoids.

Sr. No.	Active compound type	Biological Source	Compounds
1.	Alkaloids (4-6%)	Stem, root	Berberine, palmatine, jatrorrhizine
2.	Glycosides (10- 15%)	Leaf, root	Cordifolioside, tinosporaside

3.	Terpenoids (5-7%)	Flowers	Beta sitosterol, caryophyllene
4.	Flavonoids (3-5%)	Leaf, flower, stem	Quercetin, luteoiolin, kaempferol
5.	Polysaccharides (20-30%)	Stem, leaves, root	Arabinose, galactose, glucose, xylose
6.	Steroids	Stem	B-Sitosterol, Giloinsterol, Makisterone A

IV. MATERIAL AND METHODS

I. Materials:

An aerial stem of Tinospora cordifolia was collected and authenticated.
Propionobacterium Acnes Bacterial Culture, Agar medium.

II. List of Chemicals:

Sr.No.	Chemicals
	Ethanol 70%
	Xanthan gum
	Propylene glycol
	Tea tree oil
	Aloevera gel
	Triethanolamine
	Distilled water

III. List of Instruments:

Sr. No.	Name of Equipment	Purpose
1	Weighing balance	Measurement of weight
2	Magnetic stirrer	Mixing and Agitation
3	Vernier Calliper	Measuring Thickness
4	Incubator	Maintain growth and development of microorganisms
5	Autoclave	Sterlization
6	Hot air oven	Drying
7	Dessicator	Determine Weight uniformity
8	Digital pH meter	Determine pH
9	UV Spectrophotometer	Quantification,Purity determination, and identification
10	Sieve	Size seperation and ensure size uniformity

V. PROCEDURE FOR PREPARATION OF PATCH

T.Cordifolia stem was collected, cleaned and washed with water. Then, the stems were dried in a tray dry

Fig. 5 Dried stem of T.Cordifolia

Dried stems are coarsely ground; sifting was carried out using a 40-mesh sieve, weighed, and stored in airtight jars.

Fig. 6 Trituration of dried stem

The extract was prepared using the cold maceration method, and [illegible]0 ml ethanol was added to 100 g powdered T. Cordifolia at room temperature for 48 hrs.

Fig. 7 Extraction Process

Ethanolic extract of T. Cordifolia was filtered, collected and stored.

Fig. 8 Filtration

The formulation of Patch begins with mixing of Xanthan gum and aloevera gel until Homogenous mixture was formed. The ethanolic extract of plant and other excipients were added and mixed using magnetic stirrer.

Fig. 9Dissoultion of ingredients

Once the mixture was formed, it was spread uniformly on a Petri dish and without disturbance kept for evaporation at room temperature for 24 hrs.

Fig. 10 Patch formulation

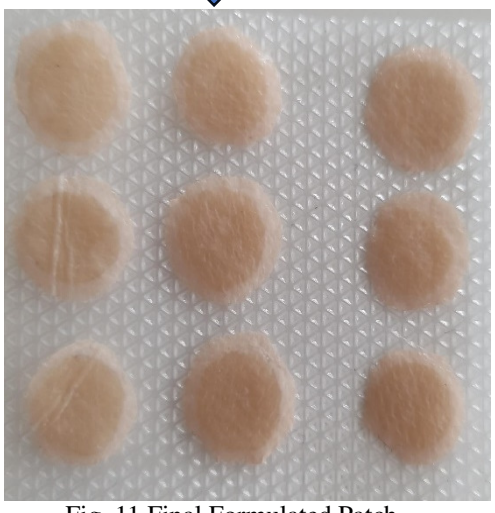


Fig. 11 Final Formulated Patch

VI. EVALUATION PARAMETERS

1) Physical evaluation of extract:

Physical assessment (color, appearance) of *T. Cordifolia* (Giloy) ethanol extract was performed.

Parameter	Observation
Color	Light Brown
Appearance	Powdered Form

2) Preliminary Phytochemical Screening:

Phytochemical screening of *T. Cordifolia* stem (ethanolic extract) was performed for the presence of alkaloids.

Sr. No.	Tests for Alkaloids	Methodology	Observations
1)	Dragendorff's Test	2-3 ml filtrate + few drops of Dragendorff's reagent	Orange brown ppt
2)	Mayer's Test	2-3 ml filtrate + few drops of Mayer's reagent	Gives ppt
3)	Hager"s Test	2-3 ml filtrate + few drops of Hager's reagent	Yellow ppt
4)	Wagner's Test	2-3 ml filtrate + few drops of Wagner's reagent	Reddish brown ppt

Fig. 12 Reagents used for test

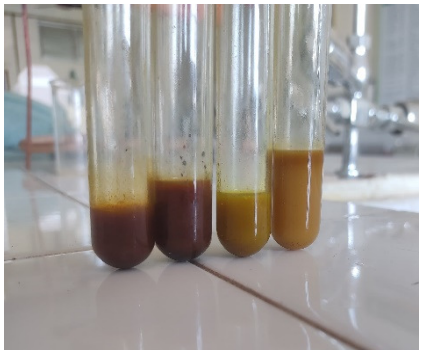


Fig. 13 Phytochemical test results

COLUMN CHROMATOGRAPHY

- Column Preparation
- Sample loading
- Elution with gradient solvent
- Fraction Collection
- Evaporate to get pure compound (Rotary Vaccum Evaporator)
- Monitoring with TLC

Fig. 14 Column Chromatography

TLC (THIN LAYER CHROMATOGRAPHY):

Thin layer chromatography (TLC) was performed on the 20 cm × 20 cm plates percolated with microcrystalline cellulose. Extracts are spotted on the plates, and TLC is allowed to run until the mobile phase reaches three-fourths of the TLC length.[22] TLC was performed using a hexane-to- ethyl acetate ratio of 4.3:1.8.

$$\text{Rf value}=\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Solute(Berberine)=2.8cm

Solvent front= 6.5cm

$$\text{Rf value} = \frac{2.8}{6.5} = 0.43$$

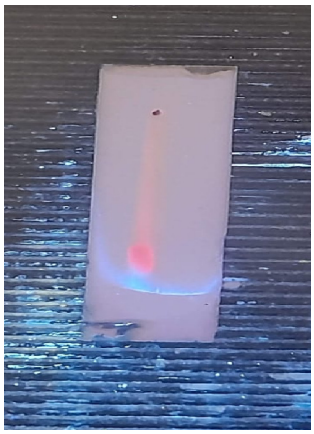


Fig. 15 TLC plate

IN VITRO TESTING:

Anti-acne activity assay of *T. Cordifolia* stem ethanol extract

The anti-acne activity of *T. Cordifolia* stem ethanol extract against *P. acnes* was tested at concentrations of 50, 100, 150, and 200 mg using the agar well diffusion method. A primary method for determining the antimicrobial activity of a compound and active substance. This method was carried out by making holes in the agar and inoculating test bacteria on Petri dishes containing agar. After that, samples were added to each hole in the cup at a predetermined concentration and incubated at 37°C for a day. Observations of antibacterial activity were carried out by reviewing clear zones indicating the inhibition zones. The inhibitory diameter was observed after incubation, and the results were recorded[15]

Sr. No.	Sample	Concentration (mg/ml)	Zone of inhibition(mm)
1)	*T. Cordifolia* stem extract	50	9
		100	12
		150	15
		200	18

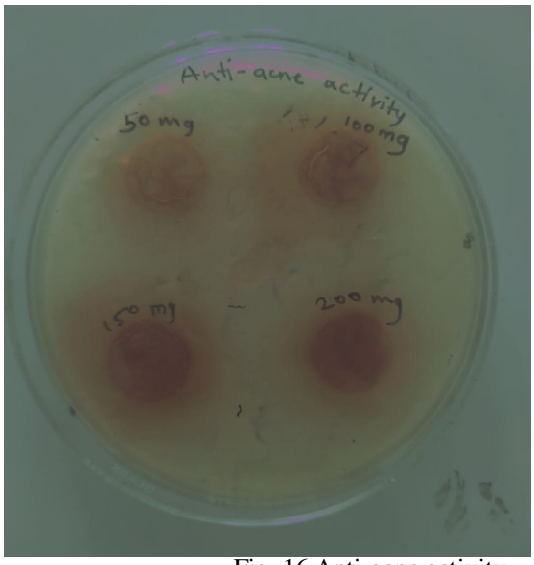


Fig. 16 Anti-acne activity

PREPARATION OF PATCH FORMULATION:

F(0) = formula without extract, *F*(1) = formula with extract, and *F*(2) = formula with extract.

Xanthan gum and aloevera gel were mixed until homogeneous mixture was formed then ethanolic extract (*T. Cordifolia*) were added, previously dissolved with 70% ethanol.

Tea tree oil and propylene glycol were then added. Once the mixture was uniform, the remaining distilled water was gradually added while stirring. Place in a Petri dish and let evaporate at room temperature for 24 hrs.

Ingredients	Functions	F(0)	F(1)	F(2)
T. Cordifolia Extract	Antibacterial	-	0.2 g	0.4 g
Xantham Gum	Polymer	1 g	1 g	1.7 g
Aloe vera Gel	Humectant	0.5 g	1.7 g	2.3 g
Tea tree oil	Anti-microbial, Flavoring agent	0.12 g	0.2 g	0.4 g
Propylene Glycol	Humectant	0.5 g	1 g	1.5 g
Distilled Water	Solvent	10 ml	30 ml	40 ml
Triethanolamine	pH stabilizer	0.02 g	0.02 g	0.04 g

IV. EVALUATION PARAMETER OF PATCH

A. *Folding resistance*

The patch is repeatedly folded the same way for the folding resistance test until broken. Then, the number of folds is considered the value of the resistance to the folding of a patch.

B. *Thickness of patch*

The patch thickness tester is used to measure the thickness individually for each of the four patch formulations to test the thickness of the patch. Measurements are taken at three different points for each formulation.

C. *Moisture absorption*

After the patch was weighed, it was kept for 24 h at room temperature. After that, it was stored at 40°C for 24 h and weighed again.[11] The formula for % Humidity to determine the percentage of moisture absorption is as follow:

$$\%\ \text{Humidity} = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100\%$$

D. *pH test*

The pH of prepared patches was determined using a pH meter.
The pH of patch was found to be 6.

E. *Drug content*

The drug release profile of a herbal patch made with Tinospora cordifolia, xanthan gum, propylene glycol, tea tree oil, Aloe vera, and triethanolamine, needed in-vitro studies (e.g., Franz diffusion cell method) and data analysis.

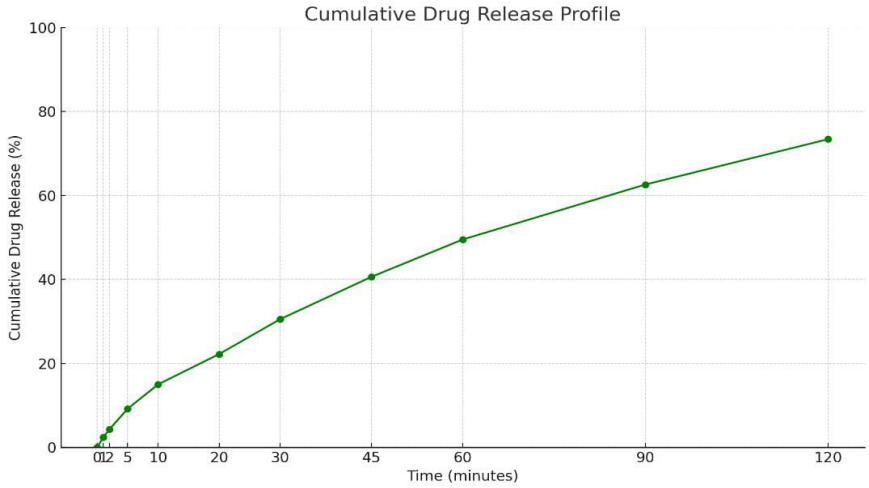
Time (min)	Absorbance (AU)	Concentration (µg/ ml)	Cumulative release(%)
0	0.000	0.00	0%
1	0.025155	0.24	2.4%

2	0.045	0.43	4.3%
5	0.095	0.92	9.2%
10	0.155	1.50	15%
20	0.230	2.22	22.2%
30	0.315	3.05	30.5%
45	0.420	4.06	40.6%
60	0.510	4.95	49.5%
90	0.645	6.26	62.6%
120	0.755	7.34	73.4%

Sr. No.	Sample	Concentration (mg/ml)	Zone of inhibition(mm)
1)	*T. Cordifolia* stem extract	50	9
		100	12
		150	15
		200	18

VII.RESULT

Sr. No.	Evaluation Parameters	Observation
1)	Folding resistance	After 7 folds, patch adhesion started to peel off and after 15 folds the material showed tearing signs
2)	Thickness of patch	0.25 mm
3)	Moisture absorption	15.15%
4)	pH	6
5)	TLC	0.43



VIII. DISCUSSION

Current study was undertaken to investigate the antimicrobial property of T. cordifolia that could serve as novel futuristic plant-based antibacterial agent for acne management Berberine is the main alkaloid constituents of the plant used for its antimicrobial property. A primary method for determining the antimicrobial activity of a compound and its active substance was carried out by invitro model through agar well diffusion method on P.Acne. Hydrocolloidal patches are chosen because they often used to speed up the healing process, it has the size of a pimple and is circular in shape. The shape is also very thin so that when used in public it is less visible. And its main property is that it sucks moisture from the pores, prevent other infections, and can prevent the formation of acne scars on the skin. Study shown that herbal Hydrocolloidal patches has not yet been discovered.

IX. CONCLUSION

Tinospora cordifolia holds significant potential as a natural ingredient in cosmetics due to its numerous therapeutic properties. The stem extract of T. cordifolia has shown antibacterial potential against major acne causing bacteria P.acne. The aim of the current research work is to prepare herbal acne patches by using herbal and natural ingredients. Formulation were prepared and evaluated for organoleptic properties and other evaluation parameters including pH, thickness, moisture absorption, folding resistance. Organoleptic tests show that F1 and F2 are transparent, while F3 is little bit light brown. All patch formulations have a thickness of 0.22-0.25mm, folding endurance 7-15 times, moisture absorption of 15.5%.Studies revealed that T. cordifolia is an excellent drug and does not have any adverse or toxic effects till now.

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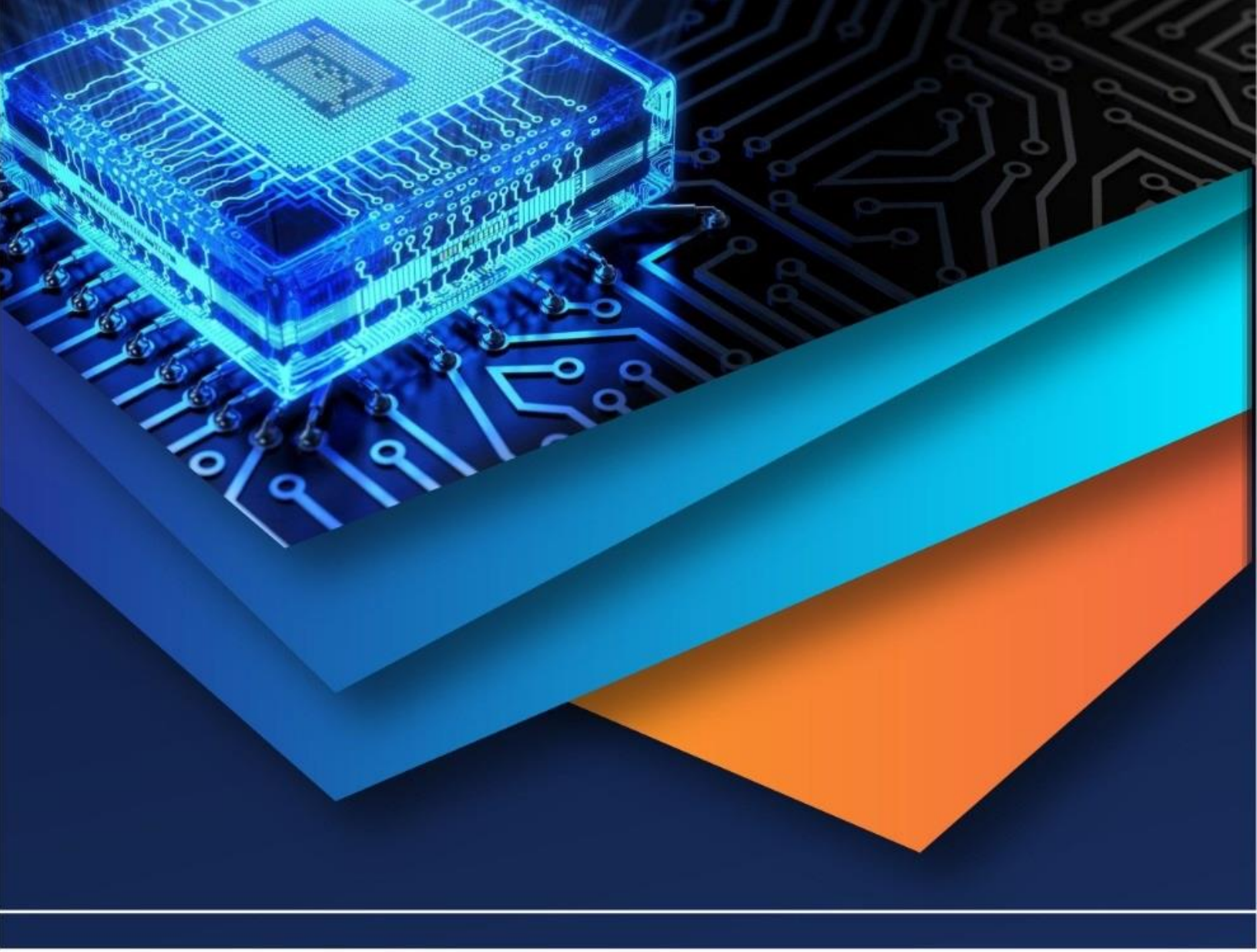

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