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Exploring the Anti-inflammatory and Antioxidant Activities of Ethanolic Flower Extracts of *Volkameria inermis*

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Abstract: ***The Research work describes the preparation and evaluation of ethanolic flower extracts from volkameria inermis, a plant recognized for its bioactive components together with flavonoids, steroids, terpenes, alkaloids, saponins, and phenolic compounds. The goal of the research work is to create a plant-primarily based remedy for inflammation. The floral extract was prepared by way of the Soxhlet method and subjected to preliminary phytochemical screening and Active compounds have been characterized with the aid of Spectroscopic Methods. The extract's antioxidant and anti-inflammatory activities were assessed in vitro and vivo. Results indicated significant antioxidant and anti-inflammatory activities. The discovery promotes Volkameria inermis as a feasible natural opportunity for artificial anti-inflammatory medications, with the gain as fewer aspect outcomes.***
Keywords: Inflammation, Flavanoids, Soxhlet, Alkaloids
Exploring the Anti-inflammatory and Antioxidant Activities of Ethanolic Flower Extracts of Volkameria inermis

I. INTRODUCTION

Inflammation is a physiological reaction that secures against microbes or tissue injury. Restoring the damaged or infected tissue is the goal of the inflammatory response. Numerous pathological conditions like cancer, heart disease, atherosclerosis, cataracts, and inflammation can be brought on by it Inflammation and oxidative stress are intimately connected and frequently happen at the same time[1-4]. Since free radicals are key mediators that initiate or maintain inflammatory processes, inflammation can be reduced by neutralizing them with antioxidants and radical scavengers. Anti-inflammatory medications must be used to control or decrease inflammation if a significant inflammatory reaction happens. In contrast to synthetic anti-inflammatory medications like Aspirin and ibuprofen, which can result in gastrointestinal problems, liver damage, renal problems, and other negative effects when taken over an extended period, natural medications are frequently linked to fewer and milder side effects. These disadvantages have led to an increase in interest in investigating natural compounds for their possible antioxidant and anti-inflammatory qualities. The evergreen, sprawling shrub *Volkameria inermis* is said to contain a variety of bioactive compounds, including cardiac glycosides, anthraquinones, flavonoids, alkaloids, phenolics, saponins, tannins, iridoids, triterpenes, sterols, steroids, carbohydrates, fixed oils, and more[5-7]. These compounds have been used traditionally for a variety of medicinal purposes, including antibacterial, antiviral, wound-healing, and anticancer effects. However, its antioxidant and anti-inflammatory activities in ethanolic flower extract of *Volkameria inermis* were not adequately explored [8,9]. The goal of the current research work is to develop a plant-based therapeutic remedy for inflammation linked to cancer, arthritis, cardiovascular disorders, etc.

II. MATERIALS & METHODS

A. Preparation of Plant Material:

The white flowers of the plant *Volkameria inermis* were collected from the girls' hostel of Aditya College, Surampalem in East Godavari District, Andhra Pradesh, India.

The flowers were washed properly and dried for 3-4 days and 20g of dried flowers were ground into a fine powder.

B. Extraction procedure[10-13]:

The *Volkameria inermis* flower extract was obtained using the Soxhlet extraction process. The flower powder was stored in a thimble inside a Soxhlet apparatus, and the solvent used was ethanol.

After assembling the Soxhlet apparatus, heat was applied to the solvent in a round bottom flask.
The soluble components from the flowers were extracted by ethanol as it boiled, evaporated, ascended into the condenser, cooled, and then returned to the thimble. This process was allowed to continue until the solvent in the thimble became colorless. To remove the solvent, the resultant extract was collected and concentrated with a Rotary Evaporator at a low pressure. The crude extract was kept in a China dish and stored in a vacuum desiccators.

C. Preliminary Phytochemical Screening[14].
The Phytochemical investigation was carried out on ethanolic flower extract of *Volkameria inermis* to detect phytochemical constituents. The ethanolic extract of flowers of *volkameria inermis* were subjected to phytochemical screening to identify the Active constituents like alkaloids,glycosides, flavanoids, saponins, etc.

D. Chromatographic Techniques:
Thin Layer Chromatography (TLC): TLC was performed to separate the Flower Extracted components based on their polarities, allowing for the visualization of Various phytochemical compounds.

E. Spectroscopic Characterization:
The isolated compounds were characterized using advanced spectroscopic techniques like FTIR.

F. Pharmacological Evaluation:[15-17]
1) Antioxidant Activity:
The Antioxidant activity of the Ethanolic Flower extract of *Volkamaria inermis* was carried out by 2 Methods.

- *DPPH Scavenging Method*: In the presence of antioxidant potential, plant extracts can donate an electron to DPPH and reduction in the DPPH free radicals measured at 517 nm. Various concentrations of plant extracts (0.3mg/ml and 0.5mg/ml)were added to 1mL of 0.1mM solution of DPPH in methanol. The absorbance was measured after a 30-min reaction under darkness at room temperature. Ascorbic acid was used as standard. Measurements were taken in triplicate The radical scavenging activity of plant extracts was calculated using the formula

  % of Radical-scavenging activity=A0-A1÷A0 ×100

Whereas A0 = absorbance of the control and A1 = absorbance of the test sample/standard. The results were reported as % inhibition and ascorbic acid equivalents (AAE, mg/g) of *Volkameria Inermis* flower extracts.

- *Phosphomolybdenum method:* This assay is based on reduction of Mo(VI) to Mo(V) by the analyte of sample and subsequent formation of green phosphate Mo(V) complex at acidic PH. 0.1 ml of various conc of plant extract (0.3mg/ml and 0.5 mg/ml) solution is combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95°C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in UV spectrophotometer. A typical blank solution contained 1 ml reagent solution and the appropriate volume of the same solvent used for the sample and is incubated under the same conditions as the rest of the sample.

2) Anti-Inflammatory Activity: It was studied by using both Invitro and Invivo methods.
Invitro Methods:

- *HRBC Method:* The blood was collected from healthy human volunteer (who had not taken any NSAIDS for 2 weeks prior to the experiment) and Mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100 and 200 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. Diclofenac (100 and 200µ g/ml) was used as reference standard and a control was prepared by omitting the extracts

- *Inhibition of Protein Denaturation method:*

Reaction mixtures were incubated in a water bath at 37˚c±2˚c for 15 -20 min. Later, it was heated at 70˚c at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 mins. Absorbance of reaction mixture before and after denaturation was measured for each concentration (10µg/ml and 1µg/ml, 0.1µg/ml) at 680nm using a colorimeter

Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula.

Percentage inhibition of protein denaturation was calculated by using the formula Percentage Inhibition = 100 – {(Abs. of Ts – Abs of Pc) / Abs. of Tc} × 100 Where Abs. of Ts = Absorbance of test solution Abs of Pc = Absorbance of product control Abs. of Tc = Absorbance of test control

Control represents 100% protein denaturation, and the result of the sample extract was compared with Standard Diclofenac sodium

- *Invivo Method: Carrageenan Induced Paw Edema Method*

➢ *Animals***:** Swiss albino mice weighing (23 ± 2g) of either sex were taken from the animal house of the Department of Pharmacology in Aditya College of Pharmacy. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines.

➢ *Method:* Edema in the right hind paw of mice was induced by an injection of 0.1 ml of 1% (w/v) of carrageenan in saline subcutaneously in the plantar side of the right hind paw of mice. The paw diameter was measured before the carrageenan injection and then each hour up to 5 times then after 24 and 48 hr. The rats were randomly divided into four groups. The first group (control group) received normal saline (0.1 ml), while the second group received the standard anti-inflammatory drug; the diclofenac (10mg/kg). The third group was treated with the extract of *Volkameria inermis* (100 mg/kg body weight). The fourth group was treated with the extract of *Volkameria inermis* (200 mg/kg body weight). The animals were pretreated 1 hr before the administration of Carrageenan.

The percentage inhibition of Oedema should be calculated by using the formula, Percentage inhibition of paw oedema = {1-(Vt / Vc)} ×100 Where Vt: Increase in paw volume in treated group Vc: Increase in paw volume in control group

III. RESULTS AND DISCUSSION

A. *Phytochemical Screening***:**

This shrub contains a variety of phytochemicals such as flavonoids, steroids, terpenes, alkaloids, saponins and phenolic compounds (Table-1).

TABLE 1. Phytochemical Screening of flower extract of *Volkameria inermis*

S. No	Compounds	Chemical tests	Ethanolic extract
01	Alkaloids	Mayers	+ve
		Hagers	+ve
		Murexide	-ve
02	Glycosides	Legal test	+ve
		Borntragers test	+ve
03	Carbohydrates	Molish test	-ve
		Fehlings	-ve
		Disaccharides	-ve
		Salwinoffs	-ve
		Iodine	-ve
04	Proteins	Biurtte	-ve
		Millons	-ve

05	Cardiac glycosides	Keller kiliani	+ve
06	Aleurone grains	Millons	+ve
07	Flavonoid's	Shinoda	+ve
		Alkaline sol	+ve
		$Fecl_3$	-ve
		NaoH	+ve
		Lead acetate	-ve
08	Volatile oil	Sudan-III red	+ve
09	Tannins	$Fecl_3$	-ve
10	Steroids	Salkowski	+ve

+ve: indicates Present; -ve: indicates absent

B. Infrared (IR) Spectroscopy

The IR spectra of the *Volkameria inermis* flower extract showed 5 characteristic absorption bands that correspond to functional groups present are identified by the presence of a broad peak at 3330 cm-1, sharp peak at 1642cm-, peak at 1445 cm-1, 2974 cm-1 indicating the presence of OH groups, carbonyl group, aromatic C=C stretching, =CH alkene and CH vibrations in essential chemical constituents like Tannins, Flavanoids, steroids, Glycosides etc. The FT-IR spectrum (Fig:1) shows a strong peak at 1041 cm-1 indicating the vibration of C-O alcohol that confirms the presence of tannins, steroids, flavonoids having -OH group.

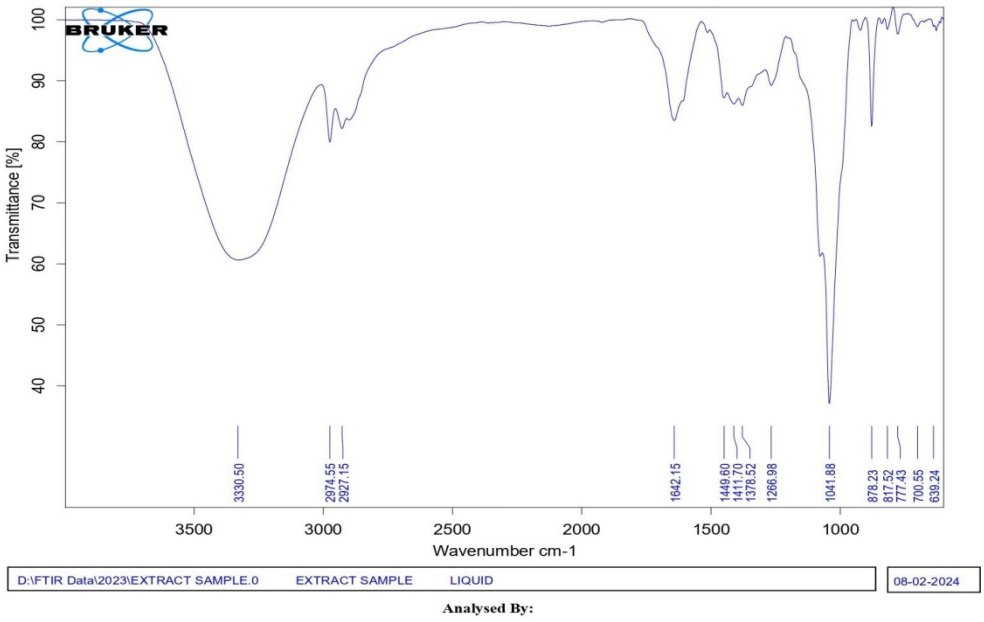

Fig. 1 IR Spectra of the Ethanolic Flower extract of *Volkameria. inermis*

C. Anti oxidant activity:

1) *DPPH Scavenging Method***:** DPPH methods were used to determine antioxidant property of the plant. The test compound of *Volkameria inermis* at conc of 10 µg/ml exhibited maximum inhibition is 84.8% when compared with standard drug Ascorbic acid 10µg/ml is 92.5%.
2) *Phosphomolybdenum Method***:** When Mo (VI) is reduced to Mo (V) by antioxidant Chemicals, a green phosphate Mo (V) complex is formed at an acidic pH, which is the basis For the assay. The measurement is carried out at 695 nm. The reducing activity of Ethanolic Flower extract is shown in Table-2, which undoubtedly shows a dose-dependent curve. An increase in methanol concentration results in an increase in the phosphomolybdenum Reduction test.

TABLE 2. DPPH, Phosphomolybdenum Methods of Test Compound in different Concentrations of ethanolic flower extract of *Volkameria inermis* and Ascorbic Acid

DPPH Method			Phosphomolybdenum Method
Sample	Concentration (µg/ml)	%Inhibition ± SEM	Absorbance ± SEM
Test compound	2.5 5 10	71.2±0.12 79.2±0.35 84.8±0.26	0.069±0.001 0.163±0.001 0.274±0.001
Ascorbic Acid	2.5 5 10	82.6±0.14 90.8±0.22 92.5±0.18	0.073±0.001 0.256±0.001 0.325±0.001

*P<0.001 when compared with standard values
Values are expressed as mean ± SEM, n=3

D. Anti-inflammatory activity:

1) HRBC Method:

One of the well-established causes of arthritic and inflammatory disorders is denaturation of tissue proteins. In vivo protein denaturation is the cause of the production of autoantigens in some arthritic conditions. Therefore, any plant substance that inhibits the denaturation of proteins may be a useful anti-inflammatory agent. The findings, which are summarized in table-3 indicate that the ethanol extract exhibited anti-inflammatory effectiveness to the common anti-inflammatory medication diclofenac sodium in a concentration-dependent manner. The Volkameria inermis flower extract shows a maximum percentage inhibition of 77% at 10µg/ml, whereas Diclofenac exhibited a maximum percentage inhibition of 82.5%.

TABLE 3. Invitro Anti-inflammatory activity of Ethanolic flower extract of *Volkameria inermis* by HRBC Method

S. No	Concentration (µg/ml)	%inhibition	
		Vol. inermis Extract	Diclofenac
1	2.5	45±0.12	67.5±0.25
2	5	65±0.24	77.5±0.14
3	10	77.5±0.18	82.5±0.35

*P<0.001 when compared with standard values
Values are expressed as mean ± SEM, n=3

2) Protein Denaturation Method:

One of the well-established causes of inflammatory and arthritic conditions is denaturation of tissue proteins. In some arthritic conditions, the production of autoantigens is due to denaturation of proteins in vivo. Therefore, any plant substance that inhibits the denaturation of proteins may have anti-inflammatory properties.The findings, which are summarized in Table-4, indicate that the ethanol extract exhibited anti-inflammatory activity to that of the common anti-inflammatory medication diclofenac sodium in a concentration-dependent manner. The *Volkameria inermis* flower extract shows a maximum percentage inhibition of 82.5% at 10µg/ml, whereas Diclofenac exhibited a maximum percentage inhibition of 85.5%.

TABLE 4. Invitro Anti-inflammatory activity of ethanolic flower extract of *Volkameria inermis* by Protein Denaturation Method

S. No	Concentration (µg/ml)	%inhibition	
		Vol. inermis Extract	Diclofenac
1	2.5	65±0.11	74.5±0.18
2	5	75.8±0.18	79.5±0.17

3	10	82.5±0.22	85.5±0.26

*P<0.001 when compared with standard values
Values are expressed as mean ± SEM

3) *Effect of ethanolic flower extract of Volkameria inermis on carrageenan-induced paw edema:* The paw edema thickness of the different pretreatment groups of rats at the fourth hour following edema induction was displayed in the photos (fig:2) and Table-5.

Volkameria inermis shows the highest Percentage inhibition of paw oedema at 4thhr at a dose of 200mg/kg by 54.9%, whereas diclofenac exhibited 59% of inhibition.

TABLE 5. In-vivo anti-inflammatory activity of ethanolic flower extract of Volkameria *inermis* by Carrageenan-induced paw oedema in rats

Treatment	Mean increase of Paw diameter (cm)				
	0 hour	1hour	2hour	3hour	4hour
Control	0.28±0.11	0.31±0.15	0.54±0.28	0.92±0.21	0.51±0.11
Diclofenac Sodium(10mg/kg)	0.19±0.21	0.30±0.22	0.46±0.15	0.66±0.23	0.21±0.25
Flower extract (100mg/kg)	0.25±0.16	0.37±0.32	0.51±0.22	0.72±0.31	0.39±0.22
Flower extract (200mg/kg)	0.22±0.25	0.35±0.33	0.48±0.25	0.61±0.36	0.23±0.25

Fig. 2. Carrageenan Induced Paw Edema Method

IV. CONCLUSION

According to the findings of this study, Ethanolic flower extract of *Volkameria inermis* has powerful antioxidant, anti-inflammatory properties, which are attributed to the presence of phenolics and flavonoids, Steroids, alkaloids. A possible source of natural pharmacological compounds that could aid in slowing the progression of a number of chronic illnesses is also suggested by these studies. However, more research is required to isolate and identify bioactive chemicals and assess them in vivo models in order to pinpoint their precise mode of action as anti-inflammatory and antioxidant agents.

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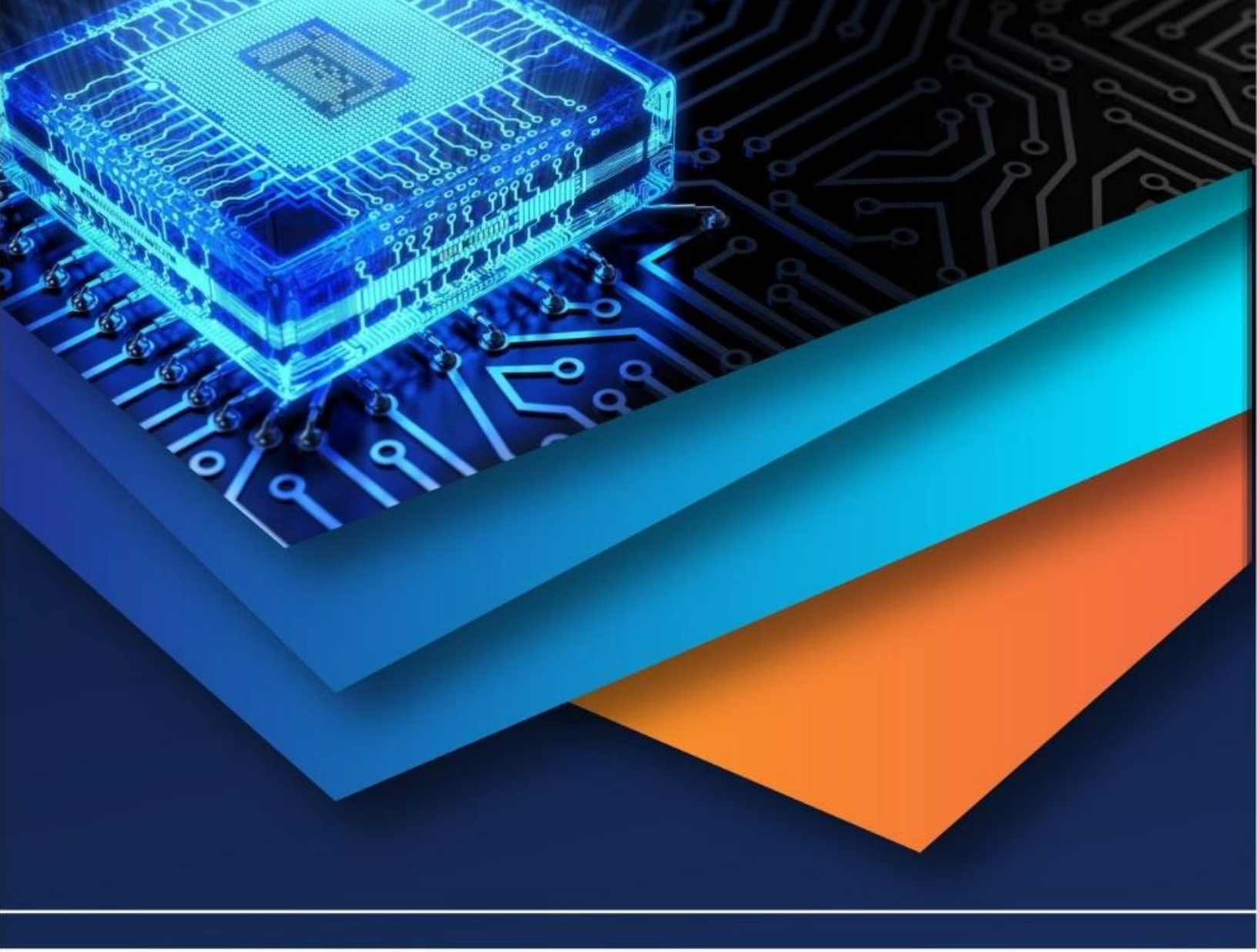

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