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Screening of Antioxidant Capacity, Anti-Inflammatory Activity, Anti-Bacterial Activity, And Phytochemical Constituents of Mangosteen Extract (Garcinia Mangostana)

Dinesh Kumar. M[1], Keerthana. K[2]
[1, 2]*Department of Biotechnology, Jeppiaar Engineering College, India*

Abstract: ***The study aims at the screening of the flesh and shell extract from mangosteen fruit with respect to its antioxidant capacity, reducing activity, anti-inflammatory activity, anti-bacterial activity and the phytochemical constituents. The mangosteen fruits were purchased and their flesh and shell were extracted separately in aqueous medium. The antioxidant capacity of the flesh and shell were assessed by their ability to scavenge free radicals such as DPPH. Their reducing activity was screened by performing the phosphomolybdenum reduction assay and the potassium ferric cyanide assay. Their anti-inflammatory activity were quantified and their anti-bacterial activity were screened by performing Antibiotic sensitivity assay. The presence of phytochemical constituents in the flesh and shell extracts were analyzed by means of confirmatory tests. The results of these assays proves the antioxidant capacity of the flesh and shell extracts of Mangosteen fruit prepared for the study.***
Keywords: Anti-bacterial activity, Anti-inflammatory activity, Anti-oxidant capacity, Flesh and shell extract, Mangosteen fruit, Phytochemical constituents, Reducing activity.

I. INTRODUCTION

The Antioxidants are the compounds that inhibit the oxidation reaction. Oxidation can produce free radicals, leading to the chain reactions that eventually cause the cell damage in the organisms. Antioxidants are commonly used as food preservatives, polymer stabilizers, and in therapeutics. In therapeutics, antioxidants are used as anti-cancer agents and in therapy for acute central nervous system injury. The use of synthetic antioxidants for these therapies impose harmful side effects on human health. The antioxidants obtained from natural products are desirable for therapeutic applications without causing any health issues. Aromatic fruits such as grapes, oranges, mangosteens are rich in their phenolic contents and hence have high antioxidant capacity. A great number of aromatic plants have been reported as having anti-inflammatory, antiallergic, antimutagenic, antiviral, antithrombotic and vasodilatory actions. The main objectives of the study are the characterization and quantification of the anti-inflammatory activity, anti-bacterial activity, phytochemical constituents of the flesh and shell extracts of mangosteen fruit; the evaluation of the antioxidant activity of the extracts by using specific assays.

II. EXPERIMENTAL PROCEDURE

A. Materials and Reagents

Fresh Mangosteen fruits (Garcinia mangostana) were purchased from a local market. Reagents such as ethanol, methanol, DPPH (1, 1-diphenyl -2-picrylhydrazyl or 2, 2-diphenyl -1-picrylhydrazyl), 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM concentrated sulphuric acid, Potassium ferricyanide, TCA (Trichloroacetic acid), 0.2 M phosphate buffer at pH 6.6, Ferric chloride solution, concentrated hydrogen chloride, picric acid solution, chloroform, acetic anhydride solution, sodium nitro prusside solution, sodium hydroxide solution, lead acetate solution, sodium chloride, nutrient agar, tetracycline were purchased and prepared with respect to the mentioned conditions. The culture samples of Staphylococcus aureus, Escherichia coli, Proteus vulgaris were purchased and stored in cold condition.

Fig. 1. Mangosteen fruits for the study

B. Extraction Procedure

The four mangosteen fruits were washed thoroughly and dried in aseptic conditions. Using a clean and methanol rinsed cutlery the shells of the mangosteens were removed carefully without piercing the flesh of the fruits. After removing the skin, the flesh of the fruits which amounts 56.70g was taken in a clean conical flask and the shells which amounts 30.30g was taken in another flask. Both flesh and shell were suspended separately in 100 mL of distilled water. The aqueous extracts of the mangosteen fruit were incubated overnight at the room temperature, which were the stock solutions. The working solutions were prepared by filtering the aqueous extracts of flesh and shell using filter paper.

Fig. 2. Flesh (left) and Shells (right) of Mangosteen fruits

Fig. 3. Stock solutions (left) and Working solutions (right) of Flesh and Shell extracts

C. Determination of Antioxidant activity by DPPH radical scavenging assay

DPPH (1, 1-diphenyl -2-picrylhydrazyl or 2, 2-diphenyl -1-picrylhydrazyl) is a synthetic stable radical. Various concentrations of the flesh and shell extracts in the range of 20 to 120 µg/mL were taken separately in two separate sets, each of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL each of methanol solution and 0.1 mM DPPH solution were added in all the tubes using a micropipette. The samples were incubated at room temperature for 30 minutes in the dark. After incubation, the absorbance of the samples having different concentrations of fruit extract were read at 517 nm using a spectrophotometer. Percentage of inhibition of the flesh and shell extracts were calculated for each sample concentrations using the formula, % of inhibition = (control - sample)/control × 100.

D. Determination Of Antioxidant Activity By Phosphomolybdenum Reduction Assay

Various concentrations of the flesh and shell extracts in the range of 20 to 120 µg/mL were taken separately in two sets, each of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL of methanol solution was added in all the tubes using a micropipette. 1 mL of the reagent, containing 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM concentrated sulphuric acid, was added in each test tubes. The samples were incubated in a water bath at 95˚C for 90 minutes. After incubation, the absorbance of the samples having different concentrations of fruit extract were read at 695 nm using a spectrophotometer. Percentage of reduction of the flesh and shell extracts were calculated for each sample concentrations using the formula, % of reduction = (sample - control)/sample × 100.

E. Determination Of Antioxidant Activity By Potassium Ferricyanide Assay

Potassium ferricyanide solution was prepared by dissolving 0.3 g of potassium ferricyanide in 30 mL of dissolved water. TCA solution and 0.2 M phosphate buffer at pH 6.6 were prepared. Ferric chloride solution is prepared by dissolving 100 µL FeCl3 in 10 mL distilled water. Various concentrations of the flesh and shell extracts in the range of 20 to 120 µg/mL were taken separately in two sets, each of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL each of methanol solution, potassium ferricyanide solution, phosphate buffer were added in all the tubes using a micropipette. The samples are then incubated in a water bath for 30 minutes. 500 µL of prepared TCA solution and 300 µL of FeCl3 solution were added in each test tubes. The absorbance of the samples having different concentrations of fruit extract were read at 700 nm using a spectrophotometer. Percentage of reduction of the flesh and shell extracts were calculated for each sample concentrations using the formula, % of reduction = (sample - control)/sample × 100.

F. Quantitative Analysis of Anti-Inflammatory activity

Saline solution was prepared by dissolving 0.45 g of NaCl in 50 mL of distilled water. Human Blood sample of group B+ was taken and centrifuged in saline solution at 7000 rpm for 15 minutes. After centrifugation, the supernatant was discarded. The above procedure was repeated twice. The pellets were then suspended in saline solution and mixed well. Thus, the human blood sample was prepared for the analysis.

Various concentrations of the flesh and shell extracts in the range of 20 to 120 µg/mL were taken separately in two sets, each of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL of saline solution and 200 µL of the prepared blood sample were added in all the tubes using a micropipette. The samples were incubated at 50˚C for 30 minutes. After incubation, the absorbance of the samples having different concentrations of fruit extract were read at 560 nm using a spectrophotometer. Percentage of hemolytic inhibition of the flesh and shell extracts were calculated for each sample concentrations using the formula, % of inhibition = (control - sample)/control × 100.

G. Determination of Anti-Bacterial Activity

The flesh and shell extracts were condensed in a hot stirrer and then dissolved in DMSO (Dimethyl Sulfoxide). Nutrient agar, having the composition of Peptone-5g, Yeast extract-3g, NaCl-5g, distilled water-1000 mL, agar-20g at pH 7, was melted by subjecting to heat. The melted nutrient agar was poured into three sterilized petri dishes in aseptic condition. The plates were kept aside for the nutrient agar to solidify. After solidification, the sample bacterial cultures such as S. aureus (Gram positive), E. coli (Gram negative), P. vulgaris (Gram negative) were taken and swabbed separately over the three different plates. In each plates, 6 wells were made in which one was the control well with no extract samples or standard, other one was filled with 30 µL of tetracycline as positive control, other two were filled with 375 µL and 500 µL of shell extract, remaining two were filled with 375 µL and 500 µL of flesh extract. The plates were then incubated overnight in aseptic condition. After incubation, the zones of inhibition of each samples in the plates were measured using zone scale.

Fig. 4. Zones of Inhibition of Flesh and Shell extracts along with standard tetracycline to the bacterial species such as S. aureus (top left), E. coli (top right), P. vulgaris (bottom)

H. Phytochemical analysis

1) *Presence of Alkaloids:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops each of concentrated HCl and picric acid were added in both the tubes. The appearance of the yellow precipitate indicated the presence of alkaloids.
2) *Presence of Terpenoids:* The flesh and shell extracts (stock solution) were taken in two different test tubes. 2 mL each of chloroform and concentrated H2SO4 were added in both the tubes. The appearance of the red ring in the middle indicated the presence of terpenoids.
3) *Presence of Steroids:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops each of acetic anhydride and concentrated H2SO4 were added in both the tubes. The appearance of the brown colour indicated the presence of steroids.
4) *Presence of Glycosides:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops each of concentrated H2SO4 and sodium nitro prusside were added in both the tubes. The appearance of the red/blood colour indicated the presence of glycosides.
5) *Presence of Flavonoids:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops of NaOH solution was added in both the tubes. The appearance of the yellow colour indicated the presence of flavonoids.
6) *Presence of Phenols:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops each of methanol and FeCl3 were added in both the tubes. The appearance of the dark green colour indicated the presence of phenols.

7) *Presence of Tannins:* The flesh and shell extracts (stock solution) were taken in two different test tubes. Few drops each of methanol and lead acetate were added in both the tubes. The appearance of the white precipitate indicated the presence of tannins.
8) *Presence of Saponins:* The flesh and shell extracts (stock solution) were taken in two different test tubes. 3 mL of distilled water was added in both the tubes. The foam formation indicated the presence of saponins.
9) *Presence of Quinones:* The flesh and shell extracts (stock solution) were taken in two different test tubes. 1 mL of concentrated H2SO4 was added in both the tubes. The appearance of the red colour indicated the presence of quinones.

Fig. 5. Phytochemical analysis samples of Flesh (left) and Shell (right) extracts after the addition of reagents

III.RESULTS AND DISCUSSION

A. Determination of Antioxidant activity by DPPH Radical Scavenging Assay

The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The absorbance of the samples, having different concentrations of the flesh and shell extracts which were read and the percentage of inhibition of the flesh and shell extracts, which were calculated for each sample concentrations, were recorded and indicated in the following tabulations.

TABLE 1
Dpph Radical Scavenging Assay For The Flesh And Shell Extracts

S. No	Sample Concentration of Flesh/Shell extract (µg/mL)	Optical Density of Flesh extract at 517 nm	% of Inhibition of the Flesh extract	Optical Density of Shell extract at 517 nm	% of Inhibition of the Shell extract
1	Control	0.649	-	0.662	-
2	20	0.438	32.51%	0.028	95.77%
3	40	0.449	30.81%	0.030	95.46%
4	60	0.184	71.64%	0.025	96.22%
5	80	0.104	83.97%	0.028	95.77%
6	100	0.055	91.52%	0.033	95.01%
7	120	0.060	90.75%	0.024	96.37%

From the tabulations, it was found that the absorbance value of the samples having different concentrations of flesh and shell extracts, decreases as the sample concentration increases. The calculated percentages of inhibition for each sample concentrations shows that the radical scavenging activity of the flesh and shell increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the flesh and shell extracts of mangosteen fruit.

B. Determination Of Antioxidant Activity By Phosphomolybdenum Reduction Assay

The antioxidant activity of samples can be evaluated by the green phosphomolybdenum complex formation. The absorbance of the samples, having different concentrations of the flesh and shell extracts which were read and the percentage of reduction of the flesh and shell extracts, which were calculated for each sample concentrations, were recorded and indicated in the following tabulations.

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TABLE 2
Phosphomolybdenum Reduction Assay For The Flesh And Shell Extracts

S. No	Sample Concentration of Flesh/Shell extract (µg/mL)	Optical Density of Flesh extract at 695 nm	% of Reduction of the Flesh extract	Optical Density of Shell extract at 695 nm	% of Reduction of the Shell extract
1	Control	0.026	-	0.026	-
2	20	0.100	74%	1.287	97.97%
3	40	0.382	93.19%	1.288	97.98%
4	60	1.198	97.82%	1.289	97.98%
5	80	1.237	97.89%	1.289	97.98%
6	100	1.722	98.49%	1.290	97.98%
7	120	2.153	98.79%	1.290	97.98%

From the tabulations, it was found that the absorbance value of the samples having different concentrations of flesh and shell extracts, increases as the sample concentration increases. The calculated percentages of reduction for each sample concentrations shows that the reducing activity of the flesh and shell increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the flesh and shell extracts of mangosteen fruit.

C. Determination Of Antioxidant Activity By Potassium Ferricyanide Assay

Potassium ferricyanide is used to determine the ferric reducing power potential of samples for the determination of their antioxidant property. The absorbance of the samples, having different concentrations of the flesh and shell extracts which were read and the percentage of reduction of the flesh and shell extracts, which were calculated for each sample concentrations, were recorded and indicated in the following tabulations.

TABLE 3
Potassium Ferricyanide Assay For The Flesh And Shell Extracts

S. No	Sample Concentration of Flesh/Shell extract (µg/mL)	Optical Density of Flesh extract at 700 nm	% of Reduction of the Flesh extract	Optical Density of Shell extract at 700 nm	% of Reduction of the Shell extract
1	Control	0.335	-	0.335	-
2	20	0.721	58.53%	0.721	58.53%
3	40	1.029	67.44%	0.843	60.26%
4	60	1.616	79.27%	1.029	67.44%
5	80	2.279	85.30%	1.616	79.27%
6	100	1.842	81.81%	1.828	81.67%
7	120	2.268	85.23%	1.842	81.81%

From the tabulations, it was found that the absorbance value of the samples having different concentrations of flesh and shell extracts, increases as the sample concentration increases. The calculated percentages of reduction for each sample concentrations shows that the reducing activity of the flesh and shell increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the flesh and shell extracts of mangosteen fruit.

D. Quantitative Analysis of Anti-Inflammatory activity

Anti-inflammatory activity is the property that reduces inflammation or swelling. The absorbance of the samples, having different concentrations of the flesh and shell extracts which were read and the percentage of hemolytic inhibition of the flesh and shell extracts, which were calculated for each sample concentrations, were recorded and indicated in the following tabulations.

TABLE 4
Analysis Of Anti-Inflammatory Activity For The Flesh And Shell Extracts

S. No	Sample Concentration of Flesh/Shell extract (µg/mL)	Optical Density of Flesh extract at 560 nm	% of Hemolytic Inhibition of the Flesh extract	Optical Density of Shell extract at 560 nm	% of Hemolytic Inhibition of the Shell extract
1	Control	1.658	-	1.408	-
2	20	1.456	12.18%	1.093	22.37%
3	40	1.188	28.35%	0.974	30.82%
4	60	1.075	35.16%	0.862	38.77%
5	80	0.961	42.03%	0.834	40.90%
6	100	0.718	56.69%	0.817	41.97%
7	120	0.201	87.87%	0.806	42.75%

From the tabulations, it was found that the absorbance value of the samples having different concentrations of flesh and shell extracts, decreases as the sample concentration increases. The calculated percentages of hemolytic inhibition for each sample concentrations shows that the anti-inflammatory activity of the flesh and shell increases with the increase in sample concentrations. It can be concluded that the anti-inflammatory activity increases with the increase in the concentration of the flesh and shell extracts of mangosteen fruit.

E. Determination of Anti-Bacterial Activity

Anti-bacterial activity is the property that inhibits the growth of specific or a wide range of bacteria. The zone of inhibition of the standard tetracycline, the different concentrations of the flesh and shell extracts for three different bacterial pathogens were measured using zone scale, recorded and indicated in the following tabulation.

TABLE 5
Analysis Of Anti-Bacterial Activity For The Flesh And Shell Extracts

S. No	Bacterial Pathogens	Zone of Inhibition (mm)				
		Standard Tetracycline	Shell Extract		Flesh Extract	
		30 µL	375 µL	500 µL	375 µL	500 µL
1	S. aureus	31	31	39	25	40
2	E. coli	23	34	35	19	22
3	P. vulgaris	27	30	33	25	26

From the tabulation, it was found that the zones of inhibition to the bacterial pathogens offered by the flesh and shell extracts were higher than the zones of inhibition offered by the standard tetracycline which is a commonly used antibiotic. Also this table indicates that the higher zones of inhibition were provided by the higher concentrations of the flesh and shell extracts. It can be concluded that the anti-bacterial activity of increases with the increase in the concentration of the flesh and shell extracts of the mangosteen fruit.

F. Phytochemical analysis

Phytochemicals are the chemical compounds produced by plants that have many beneficiary applications. The presence and the absence of some phytochemicals in the flesh and shell extracts were assessed and indicated in the following tabulation.

TABLE 6
Existence Of Phytochemicals In The Flesh And Shell Extracts

S. No	Phytochemicals	Present/ Absent in Flesh extract	Present /Absent in Shell extract
1	Alkaloids	Absent	Absent
2	Terpenoids	Present	Present
3	Steroids	Absent	Present
4	Glycosides	Present	Present
5	Flavonoids	Present	Present
6	Phenols	Present	Present
7	Tannins	Present	Present
8	Saponins	Present	Absent
9	Quinones	Absent	Present

From the tabulation, it was found that most of the major phytochemicals were present in both the flesh and shell extracts, only few were absent. It can be concluded that there is higher existence of phytochemicals in the flesh and shell extracts of mangosteen fruit.

IV. CONCLUSIONS

In the study, the antioxidant capacity, reduction activity, anti-inflammatory activity, anti-bacterial activity and the phytochemical constituents of the flesh and shell extracts from the mangosteen fruits were screened and assessed. The results of the DPPH radical scavenging assay, Phosphomolybdenum reduction assay, Potassium ferricyanide assay shows the high antioxidant capacity of the flesh and shell of the fruit. The existence of various phytochemicals were confirmed by their assessments. This study concludes that the examined mangosteen flesh and shell contain the high antioxidant, anti-inflammatory, anti-bacterial properties proving them to be a good natural source for production of desirable food preservatives and the therapeutic agents.

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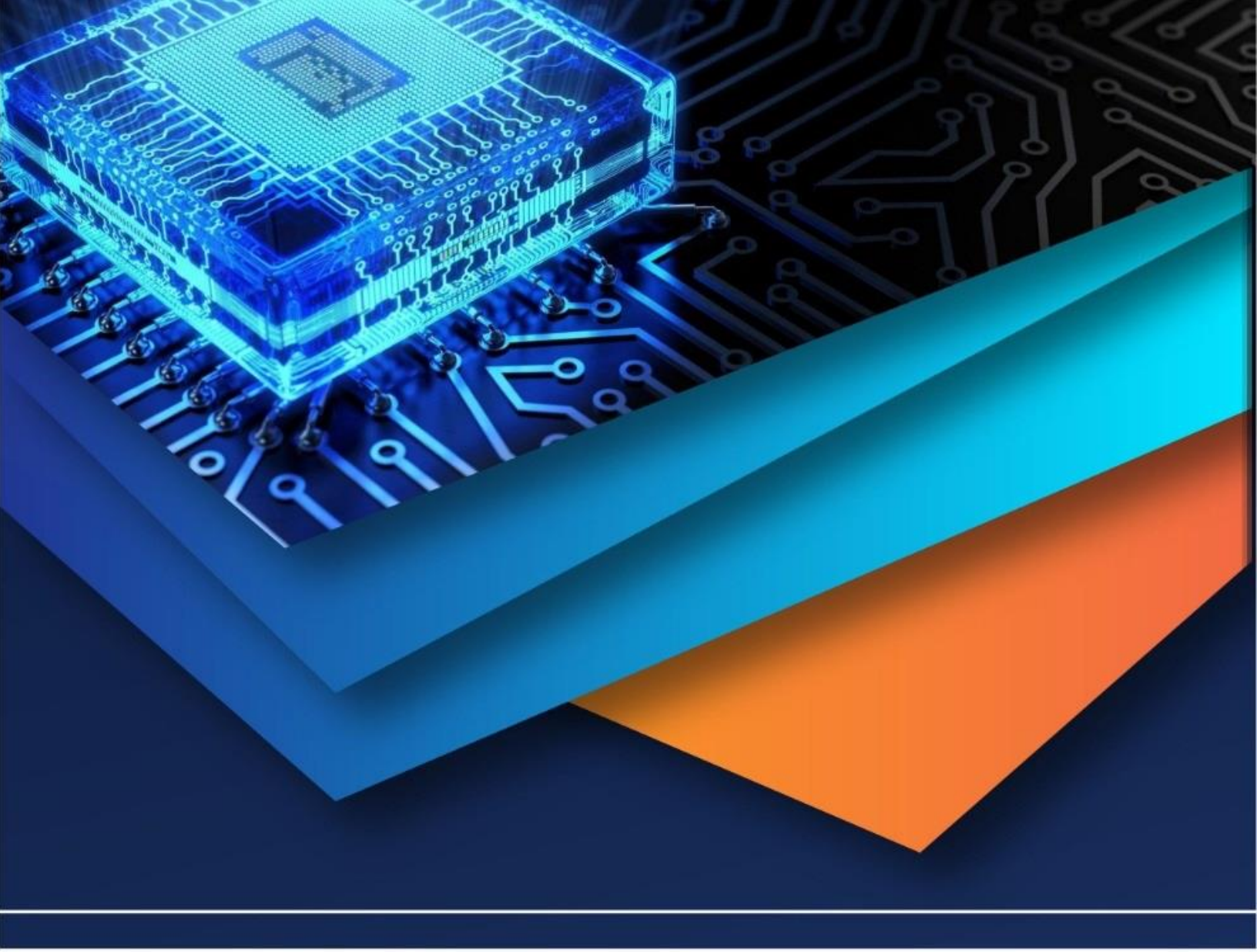

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