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Qualitative Analysis and Quantification for Total Phenolic Content (TPC) by using Different Solvent for Peels of Citrus Sinensis

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Abstract: ***Citrus sinensis is a member of Rutaceae family. Rutaceae are herbs, shrubs and trees with glandular punctate, commonly strongly smelling herbage comprising about 150 genera and 1,500 species. These are further characterized by the common occurrence of winged petioles and spines [10]. Oranges are belonging to Citrus genus and its sinensis species. For this experiment Citrus sinensis peels were used. In this experiment qualitative analysis and quantitative analysis (TPC) were done. Total phenolic content was done by using Folin Ciocalteu's technique. Extract was prepared by cold extraction method. Yield extractive value was higher in methanol extract than aqueous extract. Aqueous as well as methanol extracts of the peel revealed the presence of biochemical compounds such as alkaloids, tannins, anthraquinones, terpanoids, cardiac glycosides, flavonoids, saponins, phenols and carbohydrate and amino acid is absent in both extract.***
Keywords: Citrus sinensis, Orange peel, qualitative analysis, total phenol content, quantification.

I. INTRODUCTION

In terms of volume in production, with more than 108 million tons (FAO statistics 2006) *Citrus* ranks after banana as the world second fruit crop. Oranges are economically important fruit crops. *Citrus* fruits are main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties **[5].** It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is appreciated that other biologically active, non-nutrient compound found in Citrus fruits such as phytochemical antioxidant, soluble and insoluble dietary fibers are known to be helpful in reducing the risk of cancers, many chronic diseases like arthritis, obesity and coronary heart diseases [4]. Oranges are also rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, beta-carotene, amino acids, pectin and fiber.

A single orange have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, blood clot inhibiting and antioxidant properties. All these properties promote overall health [3]. Citrus fruits are mainly used in industries but the peels generally wasted. To utilize orange peel and pulp for the conversion into value-added product, suitable methods have to be adopted. Environmental pollution can also be reduced [1]. In the last few years, on the industrial wastes an increased attention has been focused, especially those containing residual phenols from the used plant raw material. Orange peels are one of the important dietary sources of antioxidant phenolic [8].

During the processing of fruit, orange peel is the by product. Studies show that they are good source of bioactive compounds. Every year a large amount of orange's byproducts (wastes) are formed such as peels. India produces 25 lakh of orange every year. Main orange producing states of India are Punjab, Madhya Pradesh, Andhra Pradesh, Maharashtra, Rajasthan, Assam and Karnataka. The orange peels are rich in nutrients. It contains many phytochemical. That's why they are useful. They can be useful in many drugs and food items. It is essential to find the application for these peels. During the production of orange juice and other orange products, the orange peel accumulates in the bulk and will produce environmental problems. That's why using byproduct of fruits is good thing. Tannins, terpanoids, flavonoids and saponins are present in the orange peel [7]. Citrus sinensis is effective in the management of arthritis, asthma, alzheimer's disease, parkinson's disease, macular degeneration, diabetes mellitus, gallstones,multiple sclerosis, cholera, gingivitis, optional lung function, cataracts, ulcerative colitis, crohn's disease. These health benefits are because of vitamins, especially vitamin C, Phytochemical compound like synephrine, liminoids, hesperidin flavonoid, polyphenols, pectin etc. [9].

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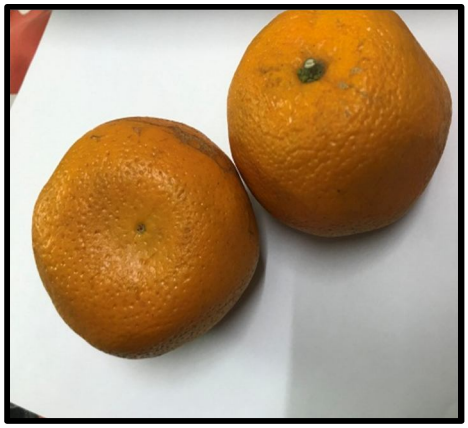

Figure 1: Citrus sinensis

II. MATERIALS AND METHODOLOGY

A. Collection of Plant Material

The selected plant material *Citrus sinensis* L. (Sweet orange) fruit peel were collected from the local market of Dhandhuka on 5 January, 2020.

Figure 2: Citrus sinensis fresh peels

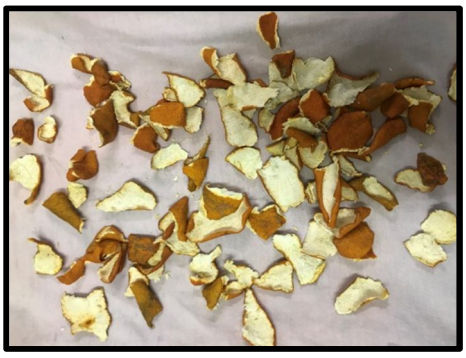

Figure 3: Citrus sinensis dried peels

B. Sample Preparation

Then selected material (Orange Peels) was dried under room temperature. Then crushed it in powder form by using grinder. Powder was store in zip lock bag for further analysis.

Figure 4: Citrus sinensis peel powder

Figure 5: Preparation of extracts

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C. Extraction of Orange Peel

10 gm of fruit peel powder was added in 100 ml of selected solvent (Aqueous (D/W) and Methanol). Then shaken it vigorously. Then keep it for soaked overnight in room temperature. After that filter it using whatmann filter paper no. 1 and filtrate was collected in petriplate. Then allowed it to dry. Kept at room temperature for the evaporation of the respective solvents.

Figure 6: Filtration

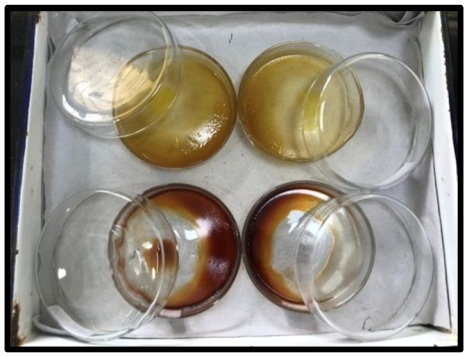

Figure 7: Peel extract

D. Yield Extractive Value

For obtaining the extractive values of plant material, the dried extracts were weighed. The yield value of each extract was calculated by using following formula:

$$\text{Yield extractive value} = \frac{\text{Extraction obtained}}{\text{Total amount of crude drug}} \times 100$$

E. Qualitative Screening

Extract was prepared by cold extraction method for qualitative and quantitative analysis. 1 mg extract was dissolved in 1 ml selected solvent. Thus, ratio of extract was 1:1 (1 mg/ml) for qualitative and quantification. Qualitative screening was performed as per the protocol followed by [1]-[6] with some modifications.

1) *Test for Alkaloids:* 2 ml filtrate was taken and it was mixed with 0.1% hydro chloric acid and 6 drops of Mayer's reagent. If pale yellow or creamish precipitates were present then it shows the presence of respective alkaloids.
2) *Test for Amino Acids:* 1 ml extract was taken in the test tube. Few drops were added of Ninhydrin reagent. Appearance of purple color indicates the presence of amino acids.
3) *Test for Tannins:* 1 ml extract was taken in the test tube. Few drops of ferric chloride were added in that and observed brownish green or blue-black coloration confirms the availability of tannins.
4) Test for Anthraquinones (Borntrager's test): 1 ml of the extract solution was taken in the test tube. Then it was hydrolyzed with diluted concentrated Sulfuric acid extracted with benzene. 1 ml of dilute ammonia was added. Appearance of rose pink coloration indicates the availability of anthraquinones.
5) *Test for Terpanoids (Salkowski Test):* 2 ml of extract was taken. It was mixed with 1 ml chloroform. 2 ml of concentrated sulfuric acid was added by the side of the test tube. Reddish brown coloration suggests the positive response for terpenoids.
6) *Test for Cardiac glycosides (Kellar-Killani test):* 2 ml of each extract solution was taken. It was treated with 1 ml of glacial acetic acid. 1 drop of ferric chloride solution was added. Concentrated sulfuric acid was carefully added by the side wall of the test tube. Appearance of brown ring in the interface indicates a presence of deoxy sugar characteristics of cardenolides.
7) *Test for Flavonoids:* 1 ml of extract was taken. 1 ml of ferric chloride was added. The formation of brown color indicates the presence of flavonoids.
8) *Test for Phenols:* 1 ml of each extract solution was taken. Lead acetate solution was added in it. Precipitate formation confirms the presence of phenols.
9) *Test for Carbohydrates (Molisch's Test):* 2 ml of each extract solution was added. Few drops of Molisch reagent were added. Some drops of concentrated hydrochloric acid were added. 1 ml concentrated sulfuric acid was added by the side of the test tube. Presence of reddish violet ring at the junction indicates the presence of carbohydrates.
10) *Test for saponin (Froth's Test):* 1 ml of extract solution was taken. 5 ml of distill water was mixed in it. It was shaken for 15 min. Formation of forms confirms the presence of saponins.

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F. Quantitative Analysis (Total Phenol Content)

The phytochemical analysis of phenolic content was performed with some modification. Using Folin Ciocalteu's technique with slight modification total phenolic estimation was done. Extracts and standard gallic acid were taken in different concentration (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml). 0.1 ml of Folin Ciocalteu's reagent was added in every test tube. Added 4.5 ml distill water and further shaken. 1 ml sodium carbonate was mixed after 5 minutes. Then blue or greenish color was shaken for 5 minutes and incubated for 30 minutes. To measure absorbance UV visible instrument spectrophotometer was used at 760 nm. In duplicates, the experiment was performed. The blank was made using distill water and methanol. Gallic acid was used as standard **[2].**

G. Statistical Analysis

The results obtained from the experiment were analyzed statistically with Standard Deviation, Standard Error.

III. RESULT AND DISCUSSION

A. Yield Extractive Value

1) Aqueous extract's yield extractive value is 14.66% and methanol extract's yield extractive value is 29.46%. Thus yield extractive value was higher in Methanol extract than aqueous extract.

Table 1: Showing yield extractive value for Citrus sinensis peel

Sr. No.	Solvent	Yield Extractive Value
1	Distill Water	14.66%
2	Methanol	29.46%

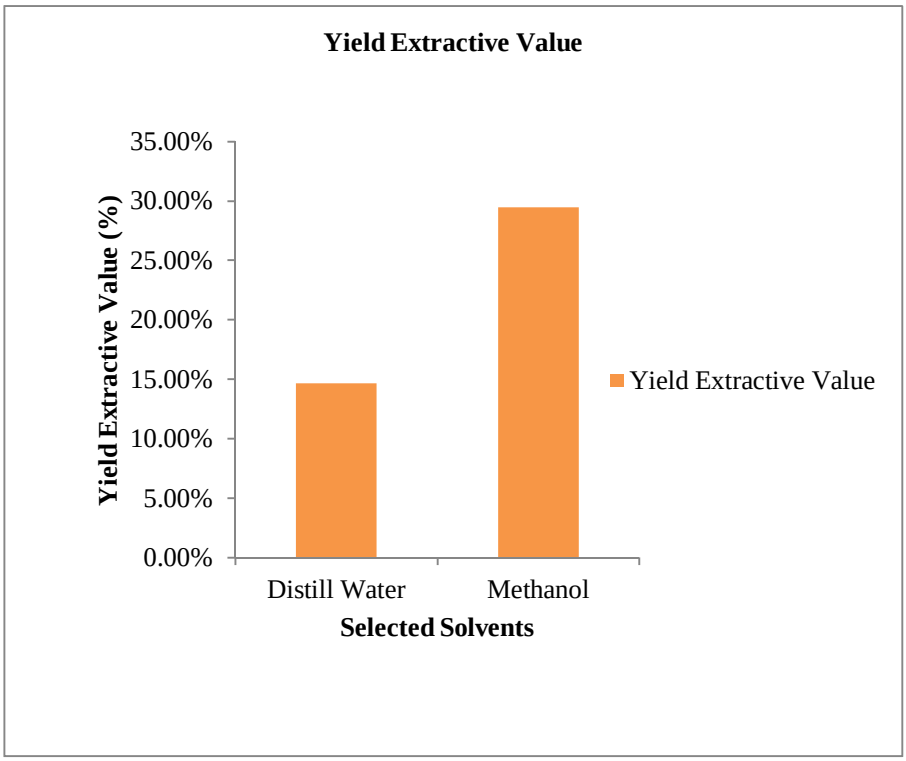

Figure 8: Yield Extractive Value of selected solvents (D/W & Methanol)

B. Phytochemical Screening

1) Extract was prepared by cold extraction method. Based on preliminary qualitative analysis, distilled water and methanol extract showed the presence of alkaloids, tannins, anthraquinones, terpanoids, Cardiac glycosides, flavonoids, saponins, phenols and carbohydrate but amino acids, flavanoids and carbohydrate was absent in distill water extract. While anthraquinones, terpanoids and cardiac glycosides were absent in methanol extract.

Table 2: Showing phytochemical screening of *Citrus sinensis* peels extract

Sr. No	Phytochemicals	Solvent	
		Distill water Solvent	Methanol ere Solvent
1	Alkaloids	++	+
2	Amino acid	-	-
3	Tannins	+	+
4	Anthraquinones	+	-
5	Terpanoids	+	-
6	Cardiac glycosides	++	-
7	Flavanoids	-	++
8	Saponins	++	+
9	Phenols	+	++
10	Carbohydrates	-	+

+ (Slightly present), - (Not present), ++ (Highly present)

C. Quantitative Analysis: Total phenolic Content (TPC)

Total phenolic content of Gallic acid for distill water extract was calculated by using the formula: y = 0.4331x + 0.0702. Total phenolic content of Gallic acid for methanol extract was calculated by using the formula: y = 0.4532x +0.0649. TPC was expressed as Gallic acid equivalents (GAE). Total phenolic content in the concentration of 0.1 to 0.5 ml of aqueous was found in the range of 0.048 to 0.289. In the concentration of 0.1 to 0.5 ml of methanol extract was found in the range of 0.032 to 0.164. The result revealed that methanol extract obtained TPC was higher than aqueous extract. The result showed that methanol extract had high TPC.

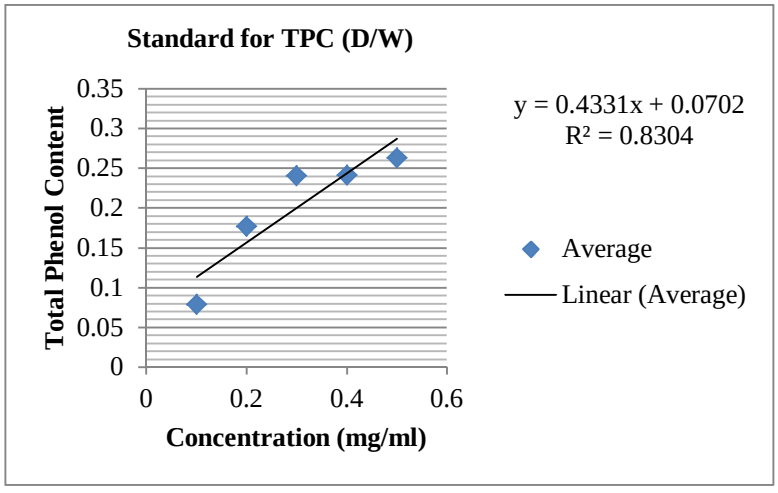

Figure 9: Standard for Gallic acid (D/W)

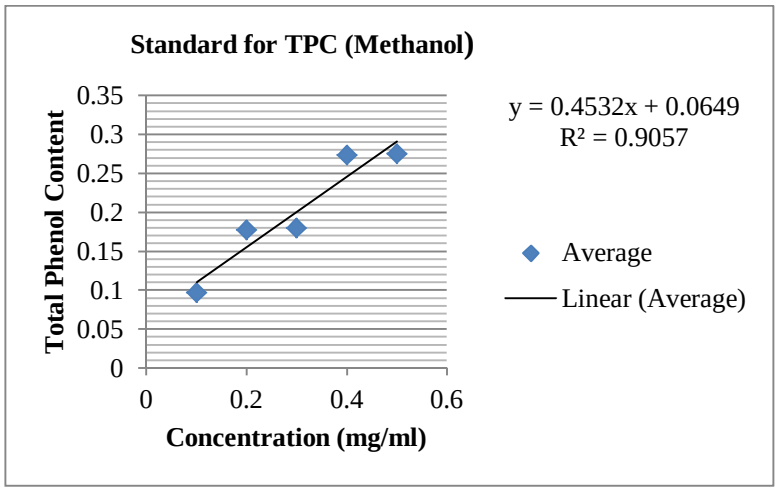

Figure 10: Standard for Gallic acid (methanol)

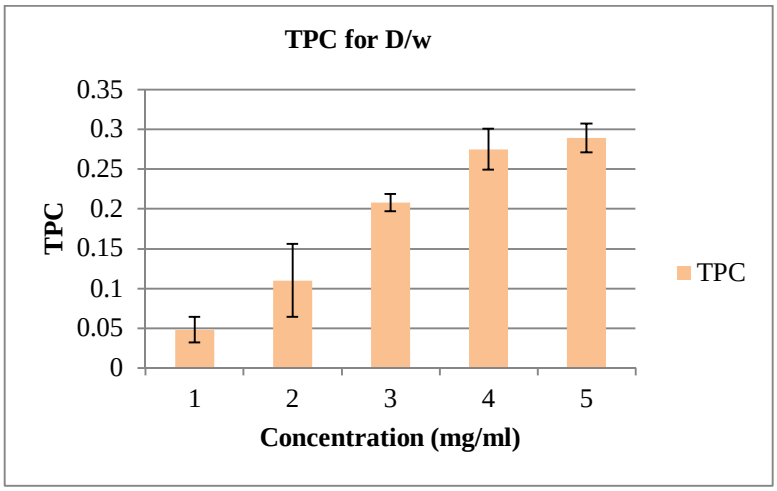

Figure 11: Citrus sinensis peels TPC (Methanol)

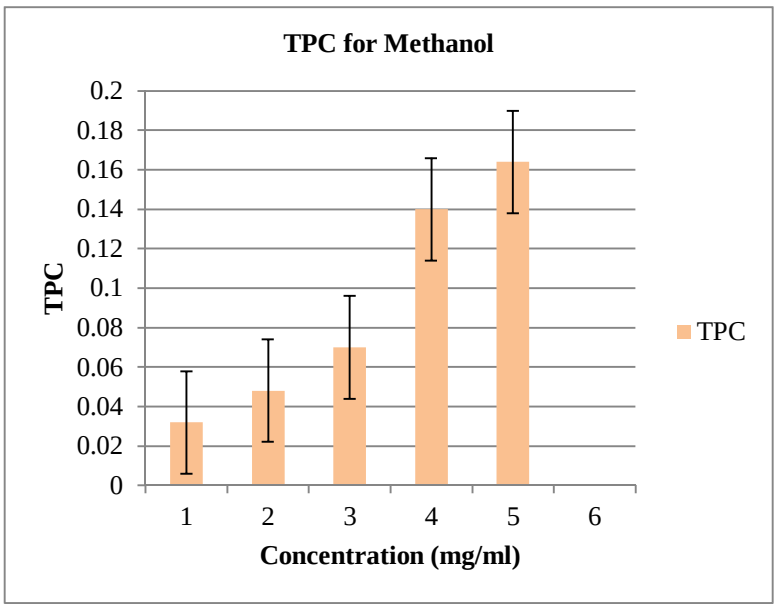

Figure 12: Citrus sinensis peels TPC (D/W)

IV. CONCLUSION

Based on this experiment it concludes that oranges are good source of bioactive compounds. Every year a large amount of orange's byproducts (wastes) are formed such as peels. During the production of orange juice and other orange products, the orange peel accumulates in the bulk and will produce environmental problems. That's why using byproduct of fruits is good thing. *Citrus sinensis* is effective in the management of arthritis, asthma, alzheimer's disease, parkinson's disease, macular degeneration, diabetes mellitus etc…Based on preliminary qualitative analysis, showed the presence of bioactive compounds such as alkaloids, tannins, anthraquinones, terpanoids, cardiac glycosides, flavonoids, saponins, phenols and carbohydrate. TPC was also obtained with good amount in *Citrus sinensis* peels.

V. ACKNOWLEDGEMENT

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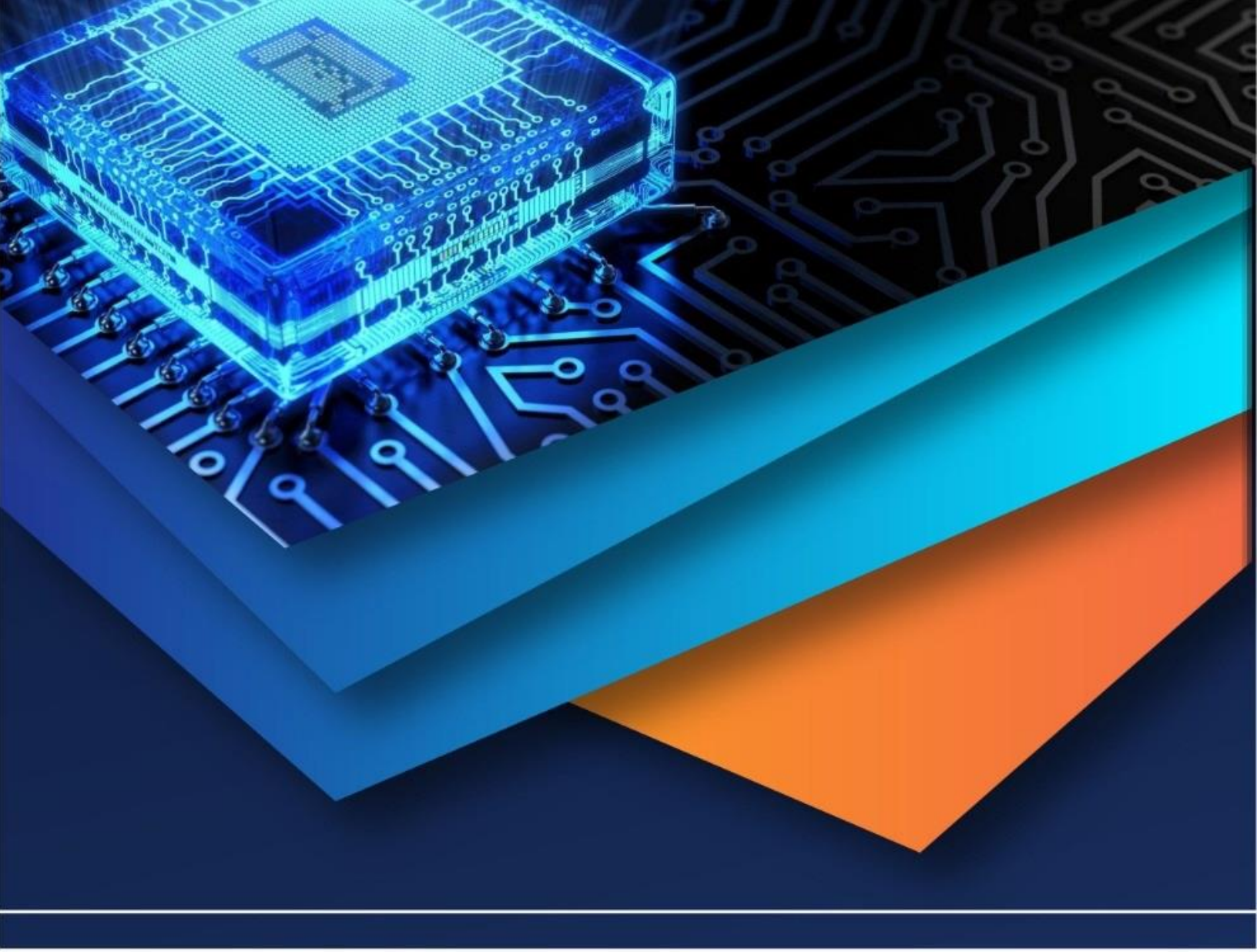

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