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Antioxidant Activity of Jackfruit based Ready-To-Cook (RTC) Curry Mixes

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Abstract:** The objective of this research was to study antioxidant activity of jackfruit based Ready-To-Cook curry mixes using seven methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, total antioxidant activity, ferric reducing capacity, ABTS radical scavenging activity, nitric oxide scavenging activity, superoxide anion radical scavenging activity and hydroxyl scavenging activity. These curry mixes were based on traditional recipes of Kerala namely, 'Avial', 'Koottu' and 'Ularth'. Antioxidant activity in the present study revealed that 'Avial mix' had the highest DPPH activity with an IC_{50} value of 33.81 µg/ml. Total antioxidant activity was found to be more for 'Avial mix' (41.44 µg/ml), followed by 'Koottu mix' (42.41 µg/ml) and 'Ularth mix' (43.45 µg/ml). 'Avial mix' showed more ferric reducing capacity while 'Ularth mix' had the least capacity in this regard. ABTS radical scavenging activities of RTC mixes ranged between 34.84-46.69 µg/ml. ABTS radical scavenging activity was observed to be higher for 'Avial mix' (34.84 µg/ml) and lower values were noted for 'Ularth mix' (40.52 µg/ml). Hydroxyl radical scavenging activity of RTC mixes was found to range between 50.55-52.55 µg/ml . 'Avial mix' showed higher superoxide radical scavenging activity with an IC_{50} value of 48.54 µg/ml and the lowest superoxide radical scavenging activity was observed in 'Ularth mix' (60.73 µg/ml). However, the highest nitric oxide scavenging activity was observed for 'Ularth mix' (14.11 µg/ml).

***Keywords:** Antioxidant activity, DPPH Radical Scavenging Activity, Total Antioxidant Activity, Ferric reducing capacity, ABTS radical scavenging activity, Nitric oxide scavenging activity, Superoxide anion radical scavenging activity and Hydroxyl scavenging activity*

I. INTRODUCTION

Diets rich in fruits and vegetables are associated with lower incidence of disease risks, including cardiovascular and carcinogenic, Kaur and Kapoor (2002). They also argue that processing or cooking can improve the health benefits of fruits and vegetables.

"Natural antioxidants" in fruits and vegetables is an area gaining increasing interest among food scientists, nutrition specialists and consumers. These natural antioxidants are claimed to promote human health and also reduce the risk of chronic diseases. The increasing awareness about the link between diet and health has consequently increased the number of scientific studies on the biological effects of these substances.

Now a day's, the consumption of fruits has increased per day due to realization of their nutritional and therapeutic effects on human health, due to the presence of phytochemicals and antioxidants. Studies have shown that a good diet with higher fruit consumption plays an important role in the prevention of chronic diseases such as heart disease, cancer, stroke, diabetes, Alzheimer's disease and cataracts (Willett 2002, Wright et al. 2008). Antioxidants are compounds capable of inhibiting the oxidation process which occur under the influence of reactive oxygen or nitrogen species. Oxidation results in the formation of free radicals, which if allowed to propagate will cause degenerative disorders. Antioxidants compact with these free radicals by intervening in the oxidative process. For the body, to remain healthy, there should be a balance between these free radicals and antioxidants.

Jackfruit is a rich source of phytochemicals, including phenolic compounds that offers opportunities for the development of value added products, such as nutraceuticals and also aid in food applications, that enhance health benefits (Umesh et al.,2010). Among the various chemical constituents, jackfruit contains useful antioxidants (Devasagayam et al.,2001). Prenylflavones isolated from Artocarpus heterophyllus serves as a strong antioxidant against lipid peroxidation (Ko et al., 1998). Carotenoids are also known to have protective function against oxidation (Mezadri et al., 2008).

Considering the various research findings, the consumption of jackfruit needs to be promoted by bringing about variety in its culinary preparations and modern lifestyle calls for Ready-To- Eat and Ready- To- Cook products. So far, various jackfruit based value added products have been standardized and their profiles analyzed for nutrients and chemicals. However, their quality assessment with respect to bio active compounds have not been attempted. Hence, the analysis of such developed products for their health benefits has become necessary. Therefore, the assessment of the antioxidant properties of jackfruit based curry mixes namely 'Avial' mix, 'Koottu mix' and 'Ularth' mix developed at the Department of Community science, was envisaged in this study.

II. MATERIALS AND METHODS

Three raw jackfruit (Koozha type) based dry mixes which were standardized at the Department of Community Science (Liji,2014), namely 'Avial mix', 'Koottu mix' and 'Ularth mix' were selected for the study. For the preparation of Ready-To-Cook curry mixes, raw jackfruit of koozha type were collected, washed and the bulbs and seeds were separated. The jackfruit bulbs and seeds were sliced into prescribed dimensions. The sliced jackfruit bulbs were blanched for 3 minutes and immersed in solution with KMS (0.2 per cent) and salt (0.5 per cent) for 30 minutes. The adjuncts used in 'Avial'mix were jackfruit bulbs, seeds, green chillies, garlic, cumin, turmeric powder and curry leaves, whereas in 'Koottu mix' jackfruit bulbs, seeds, redchillies, turmeric powder, cumin and curry leaves were blended. The adjuncts used in 'Ularth' mix were jackfruit bulbs, seeds, crushed green chillies, onion, turmeric powder and curry leaves. The details of the formulation of the three mixes are presented in Table 1.

Table 1. Proportion of ingredients in the RTC mixes

Sl No	RTC product	Ingredients	Proportion of ingredients in 100g of jackfruit
1	Avial mix	Green chillies + Garlic + Cumin + Turmeric powder + Curry leaves	3:5:1:2:5
2	Kootttu mix	Red chillies + Cumin + Turmeric powder + Curry leaves	3:1:3:5
3	Ularth mix	Crushed green chillies + Onion + Garlic + Turmeric powder + Curry leaves	2:10:5:1:5

Raw jackfruit and seeds were taken on the proportion of 70:30. The curry mixes were powdered and extracted using methanol and analysed for antioxidant activity.

A. DPPH Radical Scavenging Activity

The radical scavenging activity of the powdered curry mix extracts against 2,2 diphenyl 2-picrylhydrazyl hydrate (DPPH) was carried out using the method described by Ribeiro *et al.* (2008). The percentage inhibition of DPPH radical was calculated by comparing the result of the test with control (methanol and 1ml DPPH) using the formula

$$\text{Percentage of inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} * 100$$

B. Total Antioxidant Activity

The total antioxidant activity was determined through phosphomolybdate method (Buratti *et al.*, 2001). The curry mix extract was dissolved in phosphomolybdate reagent and incubated in water bath for 90 min. It was allowed to cool and absorbance was measured at 765 nm against the blank.

C. Ferric Reducing Capacity

Ferric reducing capacity of the powdered curry mix extracts were determined using potassium ferricyanide-ferric chloride method,0.2 ml of powdered curry mix extracts at different concentrations (25, 50, 100, 200 µg/ml) were taken and 2.5 ml of phosphate buffer (0.2 M, p^H 6.6), and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$) was added, it was then mixed and incubated at 50°C for 20 minutes to reduce ferricyanide to ferrocyanide. The reaction was stopped by the addition of 2.5 ml of 10% trichloroacetic acid (w/v) followed by centrifugation at 1000 rpm for 10 minutes. Finally, 2.5 ml of the upper layer of the mixture was mixed with distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm.

D. ABTS Radical Scavenging acTivity

ABTS radical cation was formed by reacting ABTS with potassium per sulphate. A mixture of potassium persulfate (70mM) and ABTS was allowed to stand overnight at room temperature in the dark to form ABTS radical cation, 16 hours before use, 80% methanol was used to dilute ABTS solution, from this diluted solution 100 µl was added to 2 ml of ABTS solution and the absorbance was recorded at 734nm. A standard curve was acquired by using Trolox as standard solution, at various concentrations. The scavenging activity of different concentrations of extracts against ABTS radical was estimated to ascertain IC_{50} (IC_{50} indicates the amount of antioxidant needed to oxidise the biological process by half).

E. Nitric Oxide Scavenging Activity

Sodium nitroprusside was prepared in the phosphate buffered saline and it was then mixed with different concentrations (250 and 500 µg/ml) of the powdered curry mix extracts. The mixture was incubated at 25°C for 30 minutes. A control was taken without the test compound but with equivalent amount of distilled water. Then 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent and the absorbance was measured at 546 nm and the percentage nitric oxide scavenging activity of the powdered curry mix was calculated with reference to the standard.

F. Super Oxide Anion Radical Scavenging Activity

Super oxide anion radical scavenging activity was determined based on the method described by Robok and Gryglewski (1988). In a PMS-NADH system super oxide radicals were generated via the oxidation of NADH and then it was assayed by the reduction of nitro blue tetrazolium (NBT). The super oxide radicals were generated in a reaction mixture containing 468 µm NADH solution in sodium phosphate buffer in different concentrations of methanolic extracts of the powdered curry mix samples. This was incubated at 25°C for 5 minutes and then the absorbance was recorded at 560 nm.

G. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the extracts of powdered curry mix samples were conducted using the deoxyribose method (Halliwell *et al*., 1996). The reaction mixture comprised of phosphate buffer (20 mM , p^H 7.4), deoxyribose (10 mM), hydrogen peroxide (1mM), ferric chloride (1.04 mM), and EDTA with different amounts of powdered curry mix samples (2 mM) and ascorbic acid. This solution was incubated at 37°C for 1 hour, after which 17 mM trichloroacetic acid (TCA) was added. Then it was boiled for 15 minutes, ice cooled and the absorbance was recorded at 532 nm. Distilled water was set as blank.

III. STATISTICAL ANALYSIS

All the analyses were done in triplicates. In order to obtain suitable interpretation, the generated data was subjected to statistical analysis like One-way Analysis of Variance (ANOVA) at 0.05% significant level and graphical interpretation of analyzed data was also adopted.

IV. RESULTS AND DISCUSSION

A. DPPH Radical Scavenging Activity

DPPH method is used worldwide for the quantification of free radical scavenging activity (Zhou and Yu, 2004). The data on DPPH radical scavenging activity is presented in the Table 2.

Table 2. DPPH radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	33.81
Koottu mix	34.50
Ularth mix	36.70
SE(m)	0.305
C.D	0.990

SEm : Standard error of the mean

‘Avial’ mix had the highest DPPH activity with an IC_{50} value 33.81 µg/ml, followed by ‘Koottu’ mix (34.50 µg/ml), the lowest DPPH radical scavenging activity was found in ‘Ularth’ mix (36.70 µg/ml). A study by Jagtap *et al.* (2011) stated that jackfruit wine contained 69.44±0.34 per cent of DPPH radical scavenging activity. Antioxidant activity is seen to be vary among jackfruit based products, which may due to additive effect of other ingredients and processing methods.

B. Total Antioxidant Activity

The concentration of sample that could scavenge 50% free radical (IC_{50}) was used to determine antioxidant capacity of sample compared to standard. The varieties having lowest IC_{50} had the highest antioxidant capacity. According to Blois (1992), “sample that had IC_{50}<50 ppm, was considered as very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50}>150 ppm”.

The total antioxidant activity of RTC mixes is depicted in Figure.1.

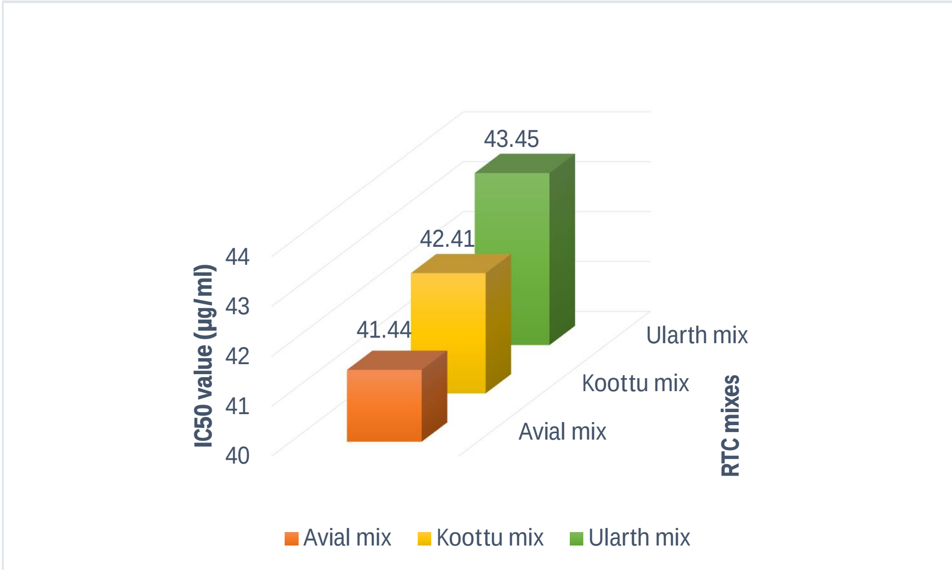

Figure 1. Total antioxidant activity of RTC mixes

Total antioxidant activity of the jackfruit based Ready-To-Cook curry mixes ranged from 41.44 to 43.45 µg/ml. The highest antioxidant activity was observed in 'Avial' mix (41.44 µg/ml) and lowest antioxidant capacity was observed in 'Ularth'mix (43.45 µg/ml). A study by Anila (2018) on different varieties of jackfruit reported that the highest antioxidant activity was observed in raw seeds of Koozha (30.35 µg/ml) and the lowest antioxidant activity was observed in raw bulbs of Sindoor (41.75 µg/ml). The antioxidant activities of the bulbs reported by Anila (2018) did not differ much with that of curry mixes of this study, because the major components of the mixes are contributed by jackfruit bulbs and seeds.

C. Ferric Reducing Capacity

The ferric antioxidant reduction assay measures the antioxidant effect of any substance in the reaction medium for its reducing capacity.

The findings revealed that 'Avial' mix (0.58 mg/100g) had the highest ferric reducing capacity followed by 'Ularth' mix (0.20 mg/100g). The lowest ferric reducing capacity was found in 'Koottu' mix (0.13 mg/100g). The difference in values were found to be significant. A study by Jagtap *et al.* (2010) found that jackfruit pulp extract showed higher ability to reduce Fe^{3+} to Fe^{2+} (1.7 Mm TEAC g^{-1} for methanolic extract).

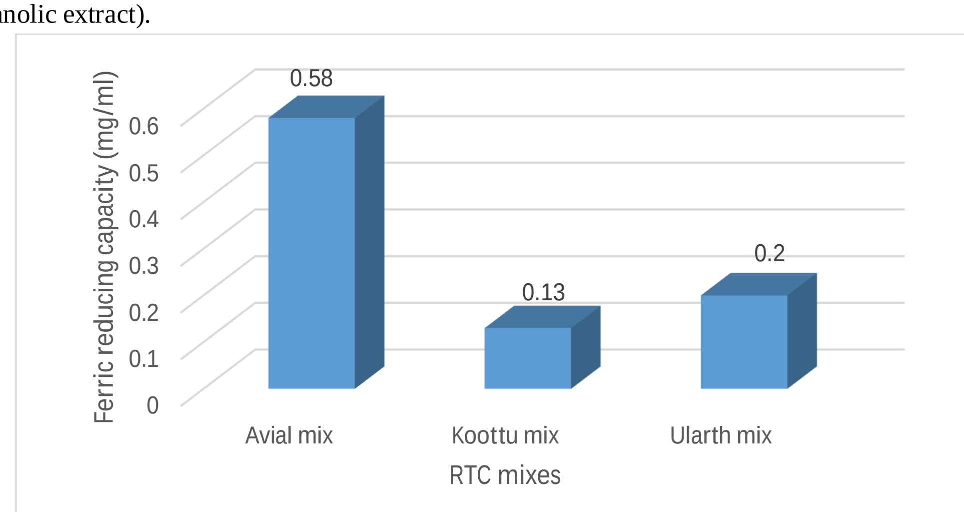

Figure 2. Ferric reducing capacity of RTC mixes

Figure 2 shows ferric reducing capacity of jackfruit based Ready-To-Cook curry mixes.

D. ABTS Radical Scavenging Activity

The ABTS assay is one of the widely used methods to evaluate the antioxidant capacities of natural products, based on the quenching of stable colored free radicals and shows the radical elimination capacity of antioxidants, even when it is present in complex biological systems, such as plant extracts or food.

Table 3. ABTS radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	36.13
Koottu mix	38.04
Ularth mix	45.56
SE(m)	0.872
C.D	2.832

‘Avial’ mix had the highest ABTS radical scavenging activity with an IC_{50} value of 36.13 µg/ml, the lowest ABTS radical scavenging activity was found in ‘Ularth mix (45.56 µg/ml). Soong and Barlow (2004) conducted a study on the ethanolic extract of defatted jackfruit seeds and in the pulp by ABTS radical scavenging assay and the results showed that fresh jackfruit seed contained an antioxidant activity of 7.4 µmol / g AAE and that the edible portion had an antioxidant activity of 3 µmol / g AAE, while the freeze dried sample showed an activity of 25.4 and 11 µmol / g AAE. The difference in the antioxidant activities could be due to extraction of antioxidants from the adjuncts.

E. Nitric Oxide Scavenging Activity

The nitric oxide or reactive nitrogen species are formed during their reaction with oxygen or superoxides, such as NO_2, N_2O_4, N_3O_4 and NO_3^-, which are very reactive. They are responsible for altering the structural and functional behavior of many cellular components.

Table 4. Nitric oxide scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	36.58
Koottu mix	30.92
Ularth mix	14.11
SE(m)	2.233
C.D	7.877

The results revealed that, there was a significant difference in nitric oxide scavenging activity of curry mixes. In case of curry mix samples, highest nitric oxide scavenging activity with an IC_{50} value of 14.11µg/ml was seen in ‘Ularth’ mix and the lowest nitric oxide scavenging activity with an IC_{50} value of 36.58 µg/ml was observed in ‘Avial’mix. A study by conducted by Basu and Maier, 2016 on in vitro antioxidant activity of seven commercially available fruits reported that jackfruit extract exhibited the highest ABTS radical scavenging activity (35.6 per cent), nitric oxide scavenging activity (87.7 per cent) and superoxide anion radical scavenging activity (55.5 per cent), processing had increased the antioxidant activity.

F. Superoxide Anion Radical Scavenging Activity

The superoxide radical is an oxygen molecule with a biologically unpaired, rather toxic electron, deployed by the immune system to kill the invading pathogen. Although superoxide anion is a weak oxidant, it leads to the generation of powerful and dangerous hydroxyl radicals and singlet oxygen, which contributes to oxidative stress and the generation of numerous degenerative diseases in humans. Table 5 shows superoxide anion radical scavenging activity of jackfruit based Ready-To-Cook curry mixes.

Table 5. Superoxide anion radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	48.54
Koottu mix	54.12
Ularth mix	60.73
SE(m)	0.584
C.D	2.059

In the present study, it was revealed that 'Avial' mix had the highest superoxide radical scavenging activity with an IC_{50} value of 48.54 µg/ml, the lowest superoxide radical scavenging activity was found in 'Ularth' mix (60.73 µg/ml). A study by Sirisha *et al.* (2014) reported that SOD activity was found to be maximum in *A. integer* (12.3±0.02) followed by *A. heterophyllus* (6.42±0.02) and minimum in *A. hircitus* (5.68±0.02.

G. Hydroxyl Radical Scavenging Activity

It is the most reactive ROS and supports the shortest half-life compared to other reactive oxygen species, and causes serious damage to adjacent biomolecules. Hydroxyl radicals have the greatest potential for electron reduction and can react with anything in the living system.

In the present study, 'Avial' mix had the highest hydroxyl radical scavenging activity with an IC_{50} value of 50.55 µg/ml followed by 'Ularth' mix (51.52 µg/ml), the lowest hydroxyl radical scavenging activity was found in 'Koottu' mix (52.55 µg/ml). Tanjung *et al.* (2014) found that hydroxyl radical scavenging activity of jackfruit extract was 36.66±1.12 per cent. Hydroxyl radical scavenging activity of RTC mixes are shown in Figure 3.

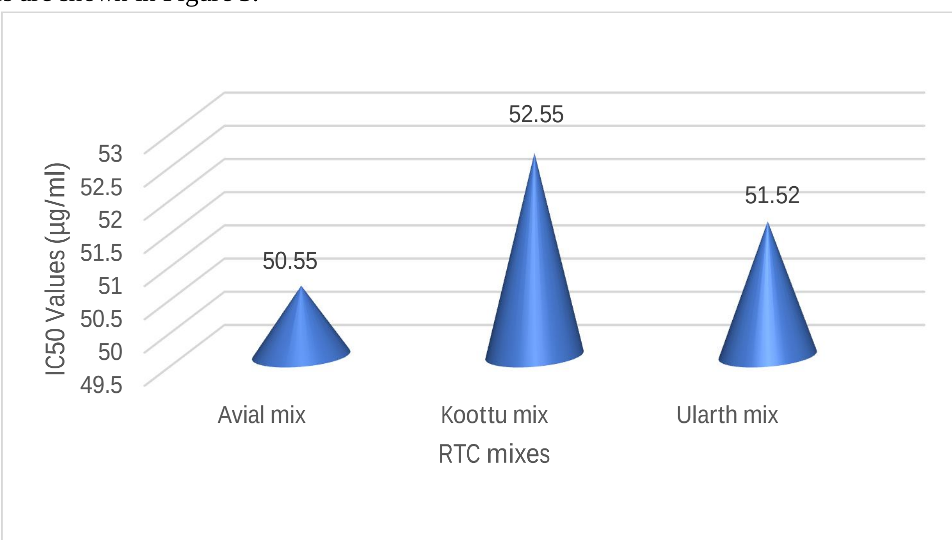

Figure 3. Hydroxyl scavenging activity of RTC mixes

V. CONCLUSION

The present study highlighted that the jackfruit based Ready-To-Cook curry mixes can serve as readymade source of various health beneficial antioxidant compounds. Antioxidant activity jackfruit based curry mixes determined by different methods indicated that it is having potent antioxidant activities and/ or free radical scavenging activity. Jackfruit is cheaper in and easily available for common man to include in their daily diet. The antioxidant compounds and antioxidant activity synergistically act to reduce the risk of degenerative diseases like cardiovascular diseases, cancer etc. Hence these products can be subscribed and promoted for commercialization as nutraceutical.

VI. ACKNOWLEDGEMENT

Department of Community Science, College of Agriculture, Kerala Agricultural University, Vellayani.

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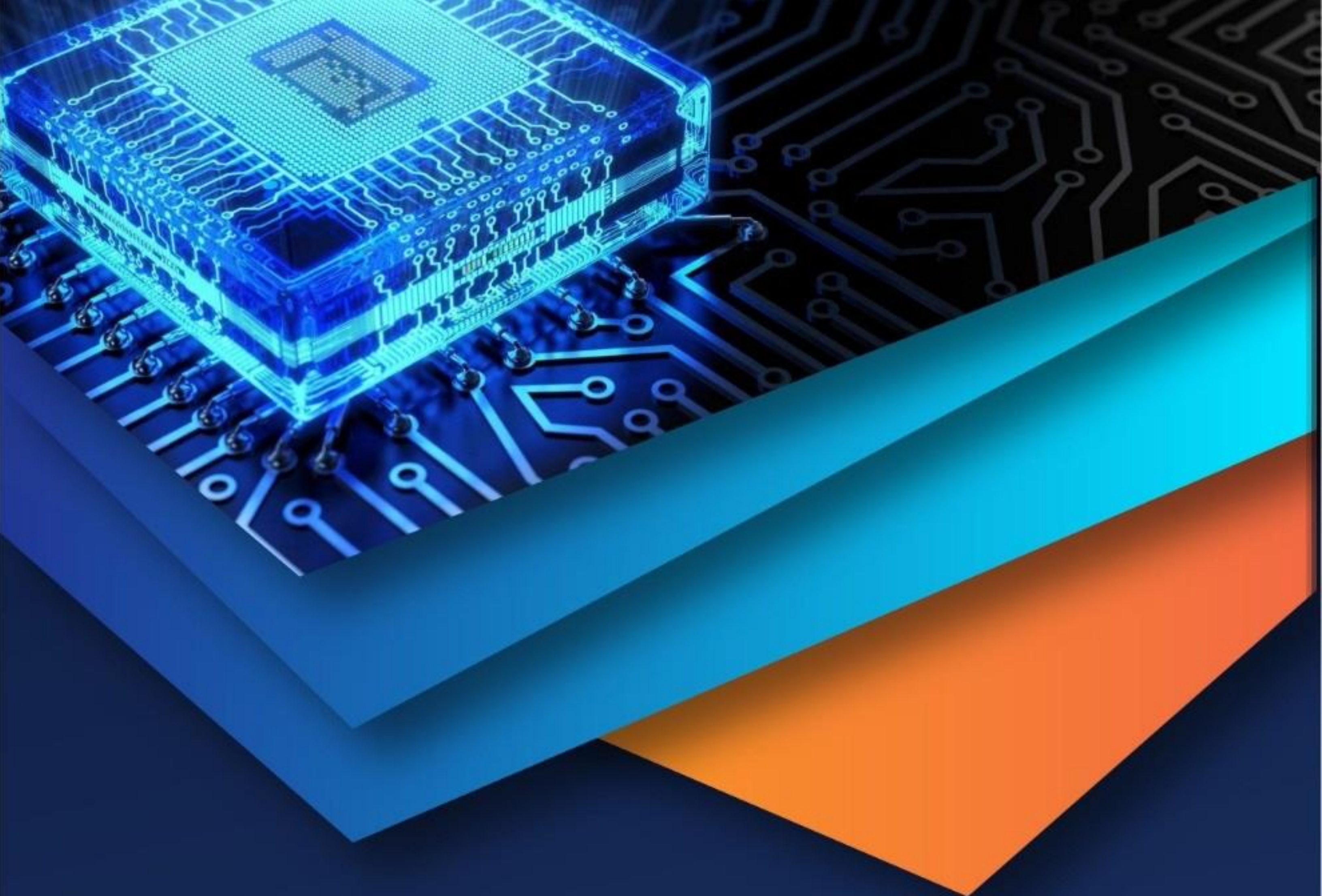

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