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A Qualitative Analysis of Caffeine from Non-Alcoholic Beverages Using UV-Visible Spectrophotometer

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Abstract: ***Caffeine is a widely consumed psychoactive compound found in various non-alcoholic beverages, including tea, coffee, and energy drinks. This study focuses on the qualitative analysis of caffeine using UV-Visible spectrophotometry, a rapid and reliable technique for detecting caffeine content. Samples were prepared using liquid-liquid separation method and analysed at their characteristic absorption wavelength, ensuring precise detection. The UV-Visible spectrophotometer, operating within a range of 200 nm to 400 nm, is sensitive to caffeine detection, providing precision and cost-effectiveness. Out of the 30 samples tested, 13 contained caffeine, despite all being labelled as non-alcoholic beverages without any indication of caffeine content. The research not only focuses on the toxicological analysis of caffeine but also highlights the importance of accurate labelling regarding its presence. The findings have significant implications for consumer awareness, regulatory bodies, and manufacturers, underscoring the need for accurate labelling of caffeine content.***
Keywords: UV-Visible spectrophotometer, Caffeine, Non-alcoholic beverages, Liquid-liquid extraction, stimulant.

I. INTRODUCTION

Beverages, any potable liquid other than water, are consumed for their stimulating effects and taste. They contain various compounds such as pigments, carbohydrates, acids, phenolic compounds, and amino acids [1]. Based on their composition and alcohol content, beverages are classified as alcoholic (e.g., beer) or non-alcoholic (e.g., fruit juice) [2]. Beverage labeling plays a crucial role in informing consumers about nutritional value, safety warnings, and caffeine content, particularly in energy drinks.

Caffeine is a naturally occurring stimulant found in coffee beans, tea leaves, cacao beans, and other plant sources. Its chemical name is 1,3,7-Trimethylxanthine ($C_8H_{10}N_4O_2$), and it appears as a white crystalline powder. Caffeine blocks adenosine receptors in the brain, reducing drowsiness and enhancing alertness. It is widely present in coffee, tea, energy drinks, and soft drinks. Excessive consumption can lead to health issues such as heart palpitations, anxiety, and insomnia [3].

Forensic significance arises in cases of caffeine overdose, where accurate labeling helps determine intake levels. Regulatory bodies require disclosure of added caffeine in food and supplements, though total caffeine content is not always mandated. Analytical techniques like chromatography, spectrophotometry, and immunoassays play a key role in caffeine detection. Studies often focus on energy drinks, coffee, and tea powders, where caffeine content is naturally high and does not always require labeling.

Extraction of caffeine is a critical step in its analysis. Liquid-liquid extraction, a widely used and cost-effective technique, separates caffeine from beverages using an organic solvent and water. Sodium carbonate is often added to stabilize the extraction process. Modern extraction methods, such as Microwave-Assisted Extraction (MAE) and Supercritical Fluid Extraction (SFE), improve efficiency but are more expensive [4].

Caffeine analysis can be performed using chemical and instrumental methods. The murexide test, a colorimetric assay, identifies caffeine based on a purplish-pink reaction. UV-Visible spectrophotometry, a widely used instrumental technique, quantifies caffeine by measuring absorbance at 273 nm. Advanced analytical methods contribute to forensic science, public health, and consumer safety by ensuring accurate caffeine detection and regulation compliance.

Regulatory bodies like FSSAI impose caffeine limits in non-alcoholic beverages, with a maximum permitted level of 300 mg/L [5]. Overconsumption, especially among youth, has raised health concerns, leading to stricter regulations. Failure to disclose caffeine content can result in legal consequences such as fines and product recalls. This study focuses on caffeine detection in non-alcoholic beverages using separation, extraction, and instrumental analysis, with potential applications in forensic investigations and public health.

II. REVIEW OF LITERATURE

Attipoe et al. (2016) conducted a study on the topic 'Caffeine Content in Popular Energy Drinks and Energy Drinks'. The use of energy drinks is high among the general population and military personnel. Previous studies have reported some discrepancies between the actual amount of caffeine in products and the amount of caffeine. The purpose of study of this research was to examine the content of caffeine in energy drinks and energy shots. Top selling energy drinks and energy shots were considered to be the samples which are collected from retail stores. For the purpose of research triplicates of each single product were purchased. The quantity of caffeine was determined by HPLC with UV absorbance after solvent extraction. When the caffeine level was below the limit of quantitation by the HPLC with UV method, liquid chromatography with tandem mass spectrometry detection was used. Some energy beverages found to have caffeine as mentioned in the label and the some which didn't shown to have caffeine were detected to have caffeine as supplement and nutrition facts.

Goldberger et al. (2006) had conducted a study on the topic 'Caffeine Content of Energy Drinks, Carbonated Soda, and Other Beverages'. In this study the caffeine content of 10 energy drinks, 19 carbonated sodas, and 7 other beverages was determined. And the variability of the caffeine content of Coca-Cola fountain soda was evaluated. Caffeine was isolated by liquid-liquid extraction and analyzed by GC with nitrogen phosphorous detection. The study was done because due to the popularity of energy drinks or functional beverages. Functional beverages are also called nutraceutical foods, which were substances considered to be a food or part of food that may give benefits on health. This caffeine content was found to be very fatal for pregnant women. The extraction procedures and instrumental parameters were carried out. Caffeine was isolated from the beverages by liquid-liquid extraction, and the final extracts were subjected to GC analysis with nitrogen phosphorous detection. Based on the calibration curve the concentration was quantified. The results were recorded. From the analysis they had reached on conclusion that the caffeine content was below the maximum allowable limits of concentration.

Johnson et al. (2011) had conducted a study on the topic 'Caffeine content of energy drink'. Caffeine is a CNS stimulant which is a methylxanthine compound. There are many other stimulants like guarana, glucuronolactone, taurine, ginseng, inositol, carnitine etc. The importance of caffeine in energy drinks, and the caffeine content of energy drinks by HPLC-DAD was the study conducted in this study. 16 energy samples were collected, in which 2 samples each of 8 brands were selected. All samples were analyzed for caffeine presence and kept sealed in the refrigerator until it is analyzed. The detection of caffeine was performed by HPLC with DAD as detector for the determination of benzoate, caffeine, and saccharin in soda beverage. Popular energy drinks like XXX, Burn, Red Bull, Cloud 9, Monster, TZINGA were analyzed. The standard curve was drawn and the detection limit was set. The results of caffeine analyzed for two different samples of 8 brands of energy drinks have been summarized in ppm. Caffeine was detected in the range about 112.23 - 314.45 ppm. Caffeine was also detected from the energy drinks which they didn't mentioned it in the label. In the research some had exceeded the limit of caffeine content, some are within the limit, and some are detected without mentioning it on the label.

Dr. C. Agatha christie and P. Ashish Willfred (2021) had done their research on the topic 'Isolation and characterization of caffeine from soft drink'. The work was presented on the isolation of naturally occurring alkaloid from carbonated beverages. Caffeine is widely used in beverages. This research was carried out to clarify level of concentration of caffeine in some of the most popular soft drinks. Caffeine serves as main ingredient in many carbonated soft drinks. FDA specifies the limit of caffeine in carbonated soft drinks. Liquid-liquid extraction is a basic technique used for the extraction purpose. Caffeine extraction was done by determining the solubility of it in the water. Ethyl acetate was used here, because ethyl acetate show more solubility in caffeine. Using separating funnel, separation of caffeine was conducted. Extraction was done in several brands and subjected to spectral analysis. Total amount of caffeine extracted were different for samples which were detected from the spectrum of UV.

Djapo et al. (2011) had made research on the topic Determination of Caffeine Content in Non-alcoholic Beverages Using HPLC Method. As the caffeine content is present in various non-alcoholic beverages, the sample population they chose were satisfied. The purpose of this study was to determine the caffeine content in beverages using reverse phase HPLC. 8 different types of non-alcoholic beverages from market in Sarajevo, B&H were chosen as sample to extract caffeine. Reverse phase HPLC was used because the most sensitive and simple method for caffeine determination was the primary objective behind the study. After the standard stock solution and sample solution preparation, the diluted solution was injected to HPLC system to determine caffeine concentration. The HPLC analysis were made. The UV spectra for the standard and sample solutions were done and the qualitative analysis were obtained. Using the data, calibration curve was constructed for caffeine standard by plotting the concentration of compound versus peak area response. The sample numbered as 2 shows the highest caffeine content. So, from the study, they had proven that HPLC technique can be used for the determination of caffeine quantitatively and qualitatively.

Vuletic et al. (2021) developed a study on 'Spectrophotometric Determining of Caffeine Content in the Selection of Teas, Soft and Energy Drinks Available on the Croatian Market'. This have a look at aimed to decide concentrations of caffeine withinside the samples of the chosen manufacturers of teas, smooth carbonated and strength beverages which might be commercially to be had at the Croatian market. The chemicals used in this study include caffeine standard (C8H10N4O2), chloroform (CHCl3) and sodium carbonate (Na2CO3). All reagents used on this have a look at have been of analytical grade and all answers have been organized with the aid of using the use of distilled water. Different samples of tea and different brands of soft and energy drinks were collected as the sample population. The UV/Vis spectrophotometer was used for the analysis of caffeine in different samples of teas, soft and energy drinks. The wavelength at which caffeine absorbs most changed into decided through scanning the variety of 190 - 400 nm. The wavelength at which caffeine absorbs maximum was found to be 274 nm. The absorbance values were used to make the calibration line for caffeine content analysis. The extraction was done by liquid-liquid separation method. And then subjected to spectroscopic analysis. The absorbance was measured at 274 nm. UV/Vis spectrophotometric approach carried out on this observe for the quantitative evaluation of the caffeine concentrations in tea and drink samples is sensitive, specific and correct. The consequences of the cutting-edge observe brought about a end that the caffeine content material must be indicated at the product labels specifically because of the extraordinary reputation and easy accessibility of caffeine-containing drinks.

Patil et al. (2018) had presented an analysis 'Extraction of Caffeine: A Review'. In the existing observe Caffeine content material of various tea and espresso samples had been studied and its miles determined that the caffeine content material varies. Caffeine and other purine alkaloids, including theobromine and theophylline, have played a major role in the long-standing popularity of non-alcoholic beverages and foods such as coffee, tea, cocoa, chocolate and a wide range of soft drinks. Tea and coffee samples were taken as the sample for their research. In order to extract caffeine from tea, numerous strategies have been used. In order to get the solid natural product into the liquid solvent, liquid or solid extraction should be used. This may be achieved through the usage of an extractor, or through surely brewing a cup of tea. So that to isolate the desired reaction compounds from the natural product, liquid/liquid extractions were used and extracted the caffeine component. Suitable solvents were used for the purpose. This study analyzed various papers and studied the methods used by various researchers to extract natural components. Different parameters were studied such as temperature, pressure and initial caffeine content for optimum yield in the previous studies. After the extraction process, iodometric titration, thin layer chromatography, and also spike test were done in this study. In the prevailing observe Caffeine content material of various tea and espresso samples had been studied and its miles located that the caffeine content material varies from 1-5%. The values generally agree well with literature quoted values of 2-5%.

Saloni Desai (2020) undertook research on the topic 'Estimation of Caffeine Content from the Soft and Energy Drinks Obtained from Regional Market by UV Spectroscopy and TLC'. This research turned into to estimate the awareness of caffeine in tender beverages and electricity beverages. The caffeine content was determined using UV spectroscopy method using dichloromethane as an extracting solvent. The goal of this examines changed into to figure out the concentration of caffeine. One of the major drawbacks of caffeine consumption is dehydration and it results the increased urine production. That's why the study was very relevant. To raise the awareness of negative effects of caffeine on human health, it is very important to conduct such researches. Because of many companies' sales their products by highlighting few advantages of caffeine, then only production and sales will increase. So that companies should be labelled the caffeine content in drinks and also its effects as well as benefits. Stock solution and aliquots were prepared and the extracted samples were introduced to sample cells. Then the sample cell was analyzed in UV Visible spectrophotometer. Buffer capacity and pH were determined. The caffeine content found from the soft drinks was more than the specified limit (FDA LIMIT). So, the conclusion from the study was the UV-Visible spectroscopy and TLC are most suitable method for determination of caffeine.

Alka Gupta and Jiya Lal Maurya (2023) carried out research on the topic 'Extraction and Analysis of Caffeine from Various Sources: A Review'. This paper includes a detailed study about the caffeine and its adverse effects. They had gone through several studies and found out that Brazil is the highest consumer of coffee in the world. Up to 400 mg of caffeine daily is safe for most healthy adults. Caffeine in powder or in liquid shape can offer poisonous tiers of caffeine, The U.S. Food and Drug Administration has cautioned. Just a single teaspoon of powdered caffeine equals around 28 cups of coffee. Such high levels of caffeine can cause serious major health problems and possibly death. Dichloromethane was used as the solvent for the extraction of caffeine. Liquid-liquid extraction method was the extraction method used in the research. The Murexide test was carried out as the confirmatory test for caffeine. The methods like thin layer chromatography, gas chromatography, iodometric back titration and spike test were carried out. The level of caffeine in all brands was found within the range.

The conclusion was coffee's caffeine content is relatively high compared to other beverages. Therefore, they had also state that caffeine is highly soluble in dichloromethane compared to other solvents.

Martin et al. (2014) presented an analysis 'Ultra-violet Spectrophotometric Determination of Caffeine in Soft and Energy Drinks Available in Yenagoa, Nigeria'. This study was carried out for the determination of pH and levels of caffeine in eight brands of carbonated and energy drinks available in local market in Yenagoa, Nigeria. In recent years, a number of the energy drinks had been introduced to the Nigerian market. This results in the further increase of health risk to consumers. A wide variety of methods have been employed including High Performance Liquid Chromatography (HPLC) being the method of choice by many researchers in determining the caffeine contents of beverages. Pure anhydrous caffeine was obtained from Coca Cola PLC, Nigeria. Different kinds of commonly consumed soft and energy drinks including Pepsi Cola, Diet Coca Cola, Mountain Dew, Coca Cola, Bullet, Power Horse, Lucozade Boost and Red Bull were purchased from the local market in Yenagoa, Nigeria. pH of beverages was determined by using Sartorius pH meter. Stock standard solution of caffeine was prepared by dissolving chloroform. The absorbance of each solution was measured at absorption maximum of 270 nm using 10mm quartz cuvette of UV Spectrophotometer. The quantity of caffeine in samples were then determined using standard curve. The curve shows a good linear relationship between the absorbance of concentration of the standard solutions. All beverages were slightly acidic. Red Bull had a lowest pH and Bullet having the highest. From the result of the study, the concentration of caffeine in the beverages were observed.

III. METHODOLOGY

A. *Materials required:*

- Sodium carbonate
- distilled water
- chloroform
- acetone
- Weighing machine
- Spatula
- Bunsen burner
- Whatman filter paper
- Glass wares

B. *Sample collection:*

All the samples collected has no labelling of caffeine in it. The samples include all three types of non-alcoholic beverages; such as refreshers, nourishers and stimulating.

Samples	Name given to samples
Sprite lemon-flavoured	S.1
7 up lemony taste	S.2
Fanta orange flavoured	S.3
Tilo power cola	S.4
Campa orange flavoured	S.5
Sapphire poko loko grape juice	S.6
Appy apple juice drink	S.7
Campa lemon flavoured	S.8
Paper boat zero sugar jamun	S.9
Paper boat jaljeera	S.10
Nescafe chilled latte	S.11
Nescafe intense cafe	S.12
Cravova classic mojito	S.13
Schweppes Indian tonic water	S.14

Dark fantasy cafe mocha	S.15
Dark fantasy chocolate shake	S.16
Hershey's milk shakes almond flavor,	S.17
Hershey's milk shakes chocolate flavor	S.18
Storia coffee shake	S.19
Milky mist chocolate milk shake	S.20
Paper boat basil sabjaseed juice	S.21
Campa cricket with electrolytes	S.22
Cavin's Belgian chocolate milk shake	S.23
Nescafe choco mocha	S.24
Winkin cow bourbon shake	S.25
Loyal mango	S.26
Slice	S.27
Mirinda	S.28
Mango treat	S.29
Alls dew	S.30

Table.I List of samples collected

C. Instrumentation

UV-Visible spectrophotometer

D. Methodology

Extraction of caffeine from non-alcoholic beverages using Liquid-liquid extraction or solvent extraction technique.

- 150 ml of sample is taken in a beaker.
- Then 2g of sodium carbonate was added to it and boiled.
- As the solution was boiled, it was filtered using Whatman filter paper and transferred to a separating funnel.
- Added 10 ml of chloroform to the separating funnel.
- The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion [22].
- The separated chloroform layer was removed to a watch glass and kept it for few minutes.

Qualitative determination of caffeine using UV-Visible spectrophotometer, the methodology for the spectral analysis of caffeine is given below:

The cuvettes are washed with acetone. Both the cuvettes are filled with distilled water to ran the baseline. After that removed the cuvettes and again washed with acetone. Each of the samples were dissolved in distilled water and filled in sample cell. Still the reference cell must be filled with the solvent used to dissolve the caffeine extracted. Then the UV-Visible spectrophotometer was ran and the graph was obtained. The cuvettes are then washed with acetone.

The obtained data from the instrument was then introduced to the origin software. Then the obtained graphs are compared and found the presence of caffeine in different beverages.

IV. RESULTS AND DISCUSSION

Total 30 samples were subjected to liquid-liquid extraction. Caffeine was extracted successfully and introduced to UV-Visible spectrophotometer. Out of 30, 13 samples were shown caffeine presence without labelling on it. Typically, caffeine content is determined by measuring the absorbance at peaks between 272 nm – 275 nm wavelength. The table.2 shows the data of different non-alcoholic beverages which is subjected to caffeine detection using UV-visible spectrophotometer. The result of presence of caffeine obtained was also mentioned on the table.

SAMPLE	SAMPLE NAME	PRESENCE OF CAFFEINE
REFRESHERS		
Sprite lemon-flavoured	S.1	×
7up lemony taste	S.2	×
Fanta orange flavoured	S.3	×
Campa orange flavoured	S.5	×
Appy apple juice drink	S.7	×
Campa lemon flavoured	S.8	×
Cravova classic mojito	S.13	×
Schweppes Indian tonic water	S.14	×
Paper boat basil sabjaseed juice	S.21	×
Campa cricket with electrolytes	S.22	×
Mirinda	S.28	×
NOURISHER		
Sapphire poko loko grape juice	S.6	×
Paper boat zero sugar jamun	S.9	×
Paper boat jaljeera	S.10	×
Nescafe chilled latte	S.11	✓
Nescafe intense cafe	S.12	✓
Dark fantasy cafe mocha	S.15	✓
Dark fantasy chocolate shake	S.16	✓
Hershey's milk shakes almond flavor	S.17	✓
Hershey's milk shakes chocolate flavor	S.18	✓
Storia coffee shake	S.19	✓
Milky mist chocolate milk shake	S.20	✓
Calvin's Belgian chocolate milk shake	S.23	✓
Nescafe choco mocha	S.24	✓
Winkin cow bourbon shake	S.25	✓
Loyal mango	S.26	×
Slice	S.27	×
Mango treat	S.29	×
STIMULATING		
Tilo power cola	S.4	✓
Alls dew	S.30	✓

TABLE.II SAMPLES AND THE PRESENCE OF CAFFEINE

Figure.1 Extracted caffeine crystal

The above two pictures show the caffeine crystals. Figure 3.0 is the extracted caffeine from Tilo power cola. The extracted caffeine samples were introduced to the UV-Visible spectrophotometer to obtain the wavelength and the absorbance. A standard UV-Visible spectrum of caffeine is taken and compared with the obtained spectra. The standard spectrum of caffeine is given below: -

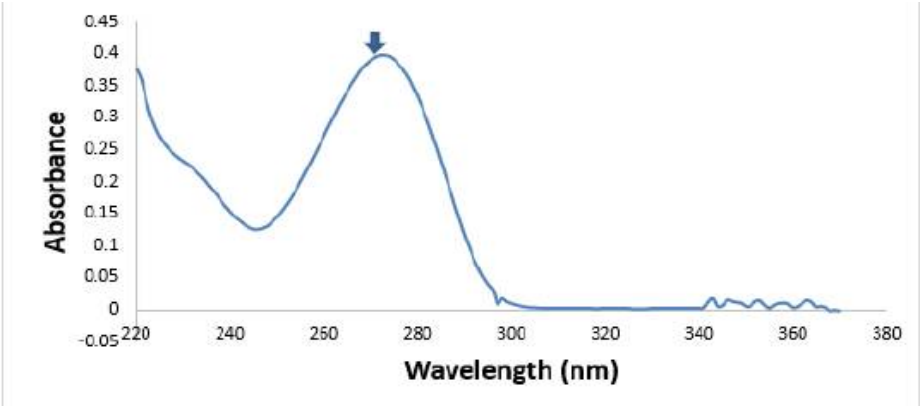

Figure.2 Spectrum of caffeine

This spectrum is taken from a research article which conducted the study on the topic 'caffeine content in energy drinks can be determined using a validated liquid-liquid extraction method coupled to UV-Visible spectrophotometry'. On this above-mentioned study the wavelength of maximum absorption determined for caffeine in acidic medium (0.1M HCl) was 273 nm from the spectrum obtained and seven energy drinks sampled from the market were successfully analyzed using this method and obtained the caffeine spectrum. (Brobbey et.al (2023))

Considering this as a standard for the reference, the absorbance values were collected. The absorbance values obtained are then given to 'ORIGIN' software, where the values are converted to graph. Origin is the data analysis and graphing software of choice for over a million scientists and engineers in commercial industries, academia, and government laboratories worldwide.[7] One of the obtained graph from origin software which gives the caffeine presence is shown below.

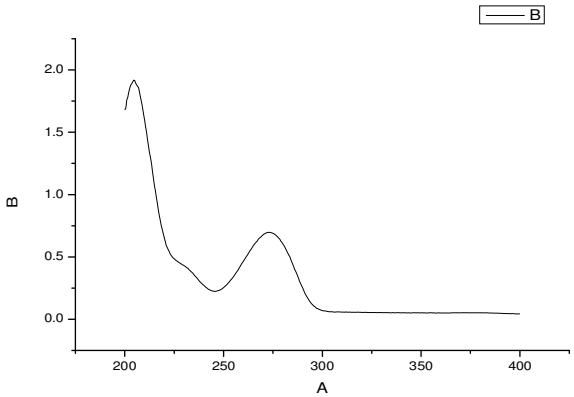

Figure.3 Spectrum of S4

V. CONCLUSIONS

This study aimed to extract and analyze caffeine from non-alcoholic beverages using liquid-liquid extraction followed by UV-Visible spectrophotometry. The method effectively confirmed caffeine presence through characteristic absorption spectra between 272–275 nm, demonstrating high specificity for a large number of samples. The results showed that beverages without caffeine labeling often contained significant amounts, sometimes exceeding those that did list caffeine.

Among the 30 samples analyzed, 13 tested positive for caffeine, with most being coffee or chocolate-flavored drinks, except for two local stimulant beverages. The study highlighted concerns regarding the lack of proper caffeine labeling, which poses potential health risks, particularly for children and pregnant women. Additionally, products explicitly labeled as caffeine-free, such as 7Up Lemony Taste, were confirmed to contain no caffeine, reinforcing the importance of accurate labeling.

The method employed was cost-effective, sensitive, and suitable for large-scale caffeine detection, making it an accessible option for routine use. Given the health risks associated with excessive caffeine consumption, including addiction and toxicity, regulatory bodies must enforce stricter labeling requirements. The FDA (Food and Drug Administration) has included caffeine in the list of substances that are mostly documented as safe and had set the maximum concentration of caffeine to be consumed.[8] Exceeding the pre-determined level is the cause of toxicity. Because of the above-mentioned health concerns arising from the consumption of caffeine, it seems appropriate that warning labels should be strictly accompany all the caffeinated beverages. Clearer labeling would ensure consumers make informed choices, ultimately promoting public health and safety.

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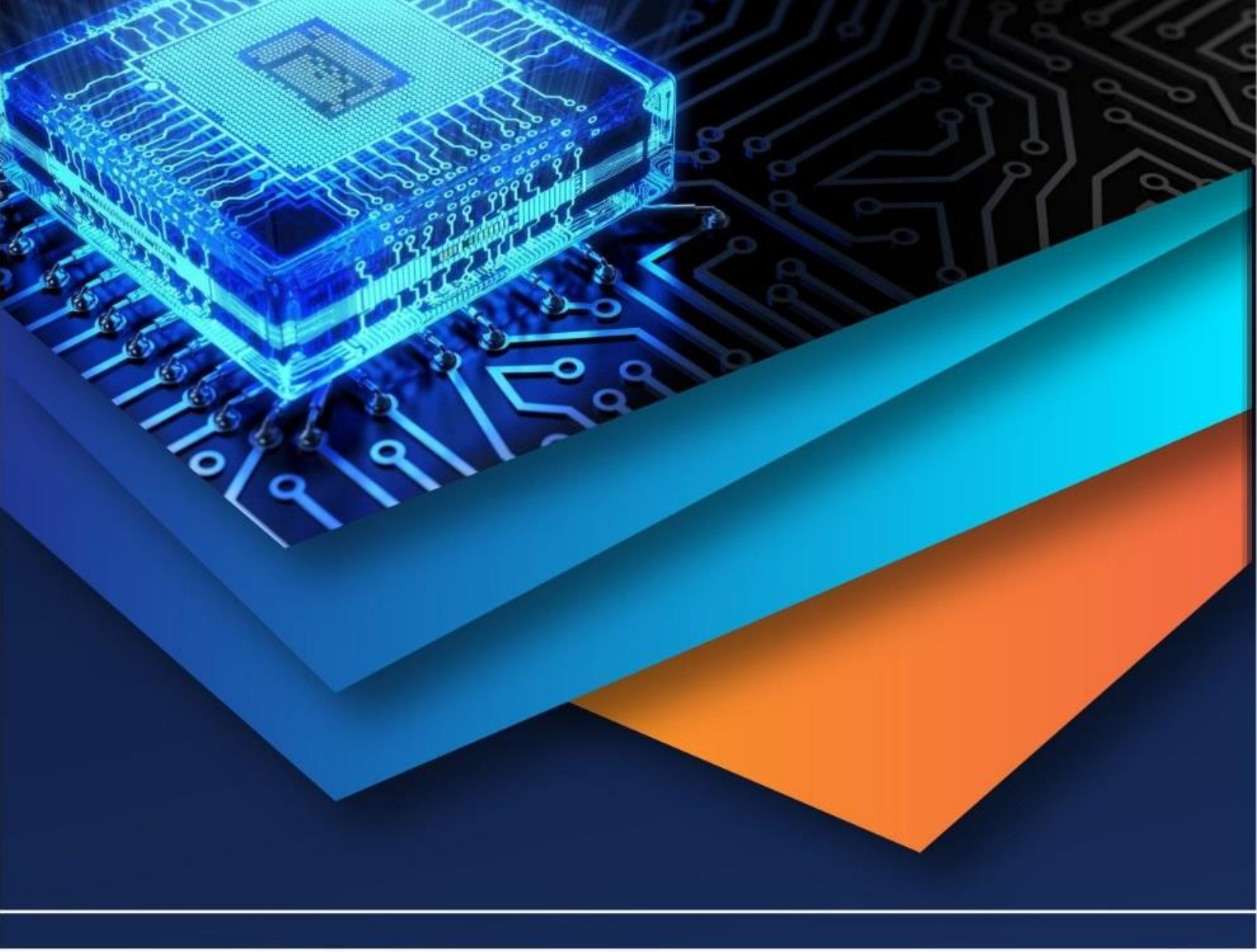

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