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In Vitro Antioxidant and Antimicrobial Properties of Methanol Extracts of Ocimum basilicum, Eucalyptus Globulus and Their Combinatio

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Abstract: ***The use of various plants combination for medicinal purposes is widely accepted in Africa. Eucalyptus globulusand Ocimumbasilicum are known for their medicinal purposes but there is no scientific evidence explaining the effect of using these plants combination. The purpose of this study was to evaluate the antioxidant and antimicrobial properties of methanol extract of Ocimumbasilicum, Eucalyptus globulus leaves and their combination. Methanol extract of the leaves and their combinations were prepared. Free radical scavenging properties were evaluated against stable 2,2-diphenyl-1-pcrylhadrzyl radical (DPPH) and hydrogen peroxide (H_2O_2). The antimicrobial properties of the leaves were determined using agar disc diffusion test. The result showed that, the combinations and the various plants differed in their scavenging activity and antimicrobial properties. The combination inhibited the growth of staphylococcus aureus for both the early morning and late afternoon extracts with high scavenging activities against both DPPH and H_2O_2. The results obtained from the study clearly indicate that Ocimumbasilicum and Eucalyptusglobulus combination are strong antioxidants and good antimicrobial agents against S. aureus.***

Keywords: Ocimumbasilicum and Eucalyptus globulus leaves, Reactive Oxygen Species (ROS), Reactive Nitrogen Species, Antimicrobial activity and Antioxidant activity.

I. INTRODUCTION

Nature has endowed human cells with adequate protective mechanisms against any harmful effects of free radicals. The superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. In a normal healthy human body, the generation of pro-oxidants in the form of reactive oxygen species (ROS) and RNS are effectively kept in check by the various levels of antioxidant defense.

Free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxyl radicals and peroxyl radicals. Many plant extracts exhibit efficient antioxidant properties due to their phytochemical constituents (Larson, 1988). Farrukhet al., (2006) investigated.

Antibiotic resistance is considered a global health concern and has been called one of the world`s most pressing public health problems (WHO, 2001). The rates of some communicable diseases have started to increase again as a result of the rise in antibiotic resistance (Levy, 1998).

Evaluation of antibacterial activities of some Egyptian medicinal plant (Alpinia galangal, Brassica oleracea, Eucalyptus globules, Inulahelenium, Ocimumbasilicum) extracts revealed that Gram-positive bacteria were more sensitive to different extracts of the various plants than Gram-negative bacteria. B. cereus and S. aureus were sensitive to all extracts except aqueous extract of elecampane, Youssef et al. (2015). The medicinal value of these plants lies in some bioactive natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable alternative antibiotics (Youssef et al., 2015).

Despite extensive research on the antioxidant and antimicrobial properties of most plants, little is known about the many tropical underutilised plants in developing nations especially Ghana. Plants such as Eucalyptus globulusand Ocimumbasilicum leaves which is a versatile perennial slender creeping herb. Extract from the leaves of Ocimumbasilicum acts principally on the digestive and nervous systems, easing flatulence, stomach cramps, colic and indigestion (Chevallier, 1992).Volatile oils of Eucalyptus are used as antioxidants and anti-inflammatory agents for skin diseases, rheumatism and as expectorant in cases of bronchitis (Guimaraeset al., 2010). Systematic investigation of extracts of these plants for their medicinal properties could provide an important input to pharmaceutical industry. Therefore, in this study, the antioxidant property and antimicrobial activities by methanol extracts of Eucalyptus globulus and Ocimumbasilicum leaves were investigated to assess the potential protective benefits of these plant against degenerative reactions induced by free radicals and microorganisms.

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II. MATERIALS AND METHODS

A. *Plant Material*

Fresh leaves of Eucalyptus globulus and Ocimumbasilicum were collected from the University of Cape Coast botanical garden, Ghana. The taxonomic identities of the plants were determined by a plant taxonomist at the Department of Botany, University of Cape Coast, Ghana. The leaf samples were washed under running tap water to remove unwanted dirt and other foreign materials. The samples were air dried under shade until no moisture left. The dried samples were ground into powder using a blender.
Gram-positive and negative bacteria from Escherichia coli and Staphylococcus aureus, were obtained from University of Cape Coast Hospital. All strains were grown on nutrient broth and were kept at the clinical laboratory.

B. *Preparation of plant extracts.*

1) *Methanol extraction:* The methanol extract was prepared by soaking 50 g of powdered sample (Eucalyptus globulusand Ocimumbasilicum) in 200 ml of methanol (70 %) for 72 h at room temperature (35°C). The mixture was then filtered using Whatman filter paper No 1. The filtrate was concentrated under reduced pressure using rotary evaporator at temperature of 46°C. The resulting extract was weighed and stored in airtight bottles at room temperature for further used.

C. *Determination of free radical scavenging activity*

1) *Determination of Hydrogen Peroxide radical scavenging activity:* The ability of the leaf extracts to scavenge hydrogen peroxide was determined according to the method of Ruch*et al.*, (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts of various concentrations in distilled water were added to a hydrogen peroxide solution 40 mM. Absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing the phosphate buffer without hydrogen peroxide.

All experiments were performed in triplicates. The % inhibition of hydroxyl radical scavenging was calculated by using the formula:

$$OH^{\cdot-}scavenged\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

2) *Determination of1, 1 Diphenyl-2, picrylhydrazine (DPPH) - free radical scavenging activity:* DPPH radical scavenging activity was assessed according to the method of Shimada et al [16]. The reaction mixture contained 1.0 mL of various concentrations (0.02-0.10 mg/mL) of extracts and standard (gallic acid) and 1.0 mL of DPPH solution (0.135 mM). The mixture was shaken vigorously and left in a dark for 30 minutes. The absorbance was measured at 517 nm against a reagent blank containing only methanol. All experiments were performed in triplicates. The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

$$DPPH\ scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

3) *Determination of Antimicrobial Activity:* Muller Hinton Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs approximately 6mm in diameter were soaked with 15µL of the plant extract and placed in the previously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other. The agar plates were then incubated at 37 ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the Cefotaxime and Ciprofloxacin used as positive control and water as negative control (NCCLS, 1997)

III. RESULTS AND DISCUSSION

A. *Antioxidant activity*

The two different extract for the various harvesting stages, the early morning and the late afternoon, for both the DPPH and H_2O_2 were observed to be concentration dependant. This observation was not consistent with work done by (Farukhet al.,2006: Moghadamet al., 2011; Ghaffaret al., 2015,). Moghahadamet al. (2011), in their work, observed that the plant extract that was harvested later recorded low bioactive components compared to the extract from the first harvest. This observation was contrary to what was observed in this study. Both the highest concentration 0.1 mg/mL of the morning and the afternoon plants leaves extracts recorded as high as 61 % for extract for all the leaves extracts but that of Ocimum and the combination drop in their scavenging activity to as low as 30 % for Ocimumbasilicum for the early morning harvesting. This observation could be due to the fact that, as

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the day length increases temperature also increases, the plant produces more secondary metabolite to aid or help prevent oxidative stress hence the observed drop in scavenging activity. Oxygenated monoterpenes which act as antioxidant are produced by plant in warmer conditions to protect plants and regulate temperature (Adams, 2008).

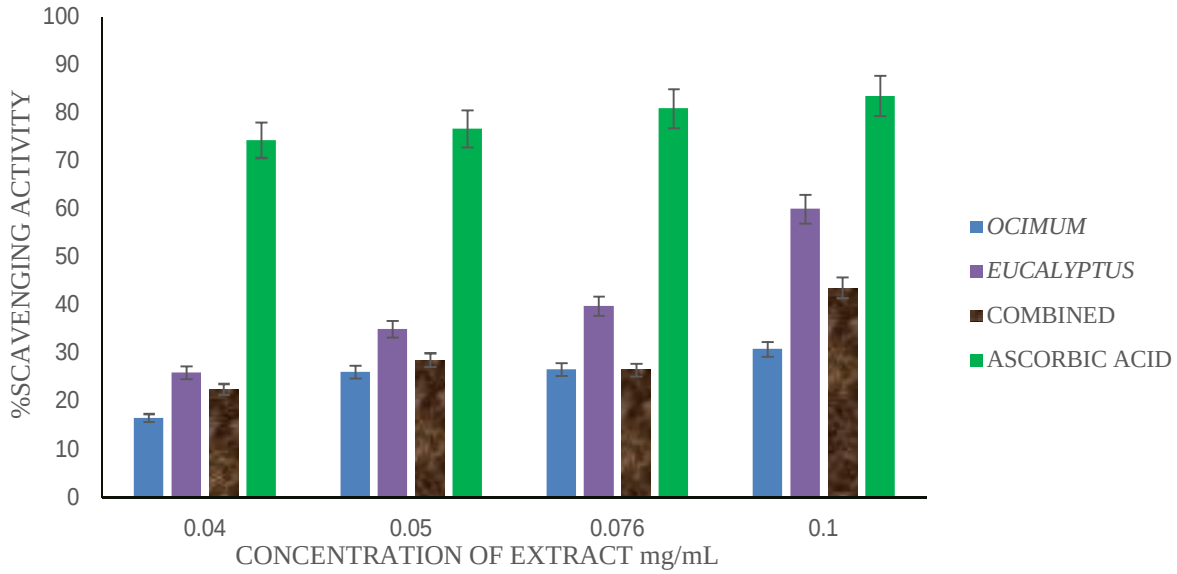


Fig.1: Free radical scavenging of DPPH by methanolic leaves extract of Ocimum basilicum, Eucalyptus globulus and combination of the two plants in morning.

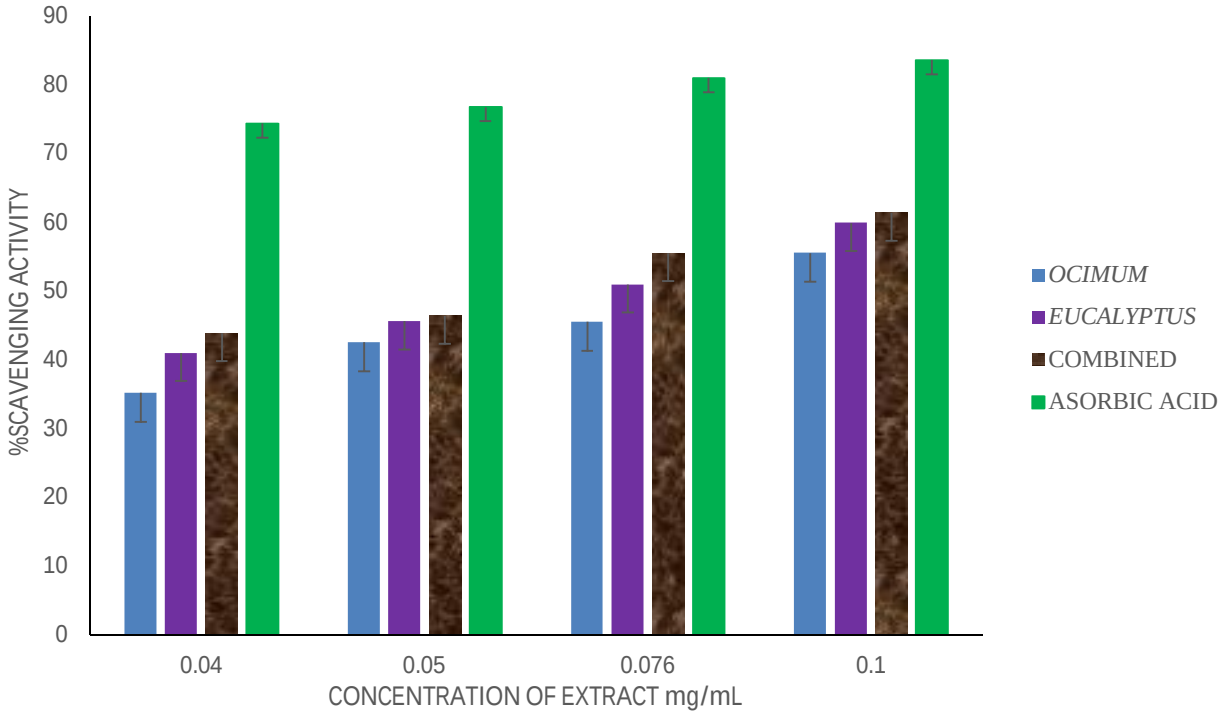


Fig.2: Free radical scavenging of DPPH by methanolic leaves extract of*Ocimumbasilicum*, *Eucalyptus globulus* and their combination in the afternoon.

The combination for the morning extract recorded scavenging activity of 45 % whiles that of the afternoon recorded 63.1 % for same extract concentration of 0.1 mg/mL. The combination extract from leaves *Ocimum* and *Eucalyptus* although showed good

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scavenging activity, their scavenging among the three different extract concentration could be described as indifferent, additive, antagonistic, effects (Burt, 2004: Delgado *et al*., 2010). An additive effect is observed when the combined effect is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less when they are applied together than when individually applied. Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects (Burt, 2004) while the absence of interaction is defined as indifference. This observation can be explained due to the presence of common bioactive component in the two plant extracts Linalool, carvarol (Farukee*t al.*, 2011; Henri *et al.*, 2012; Ghaffar*et al*, 2015). Most studies attributed additive and synergism effects to phenolic and alcohol compounds.

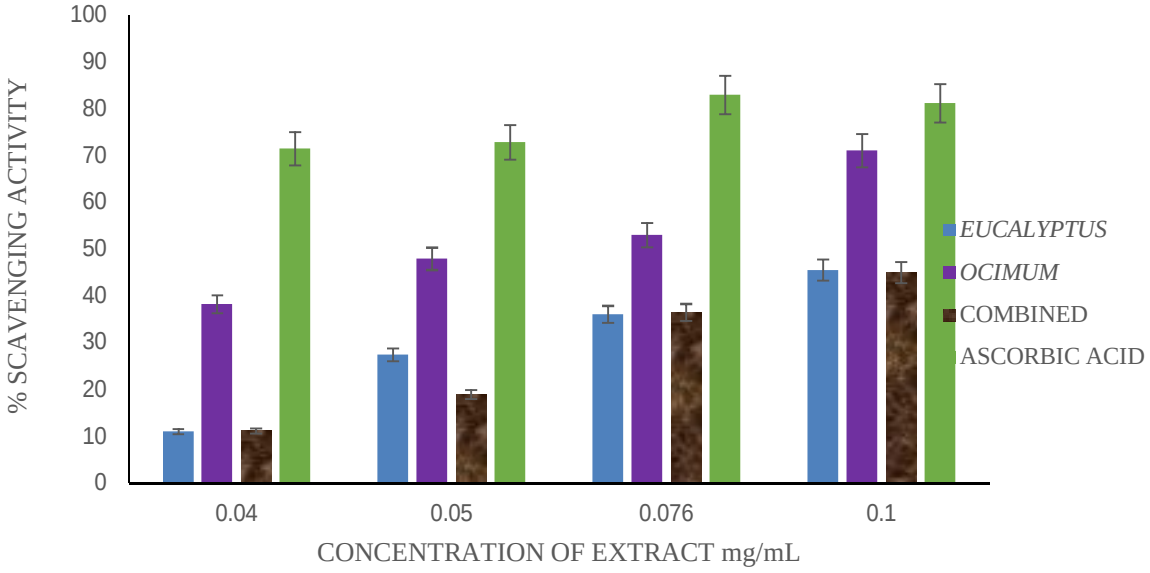


Fig. 3: Free radical scavenging of H_2O_2 by methanolic leaves extract of *Ocimum basilicum,Eucalyptus globulus* and their combination (M) in the morning

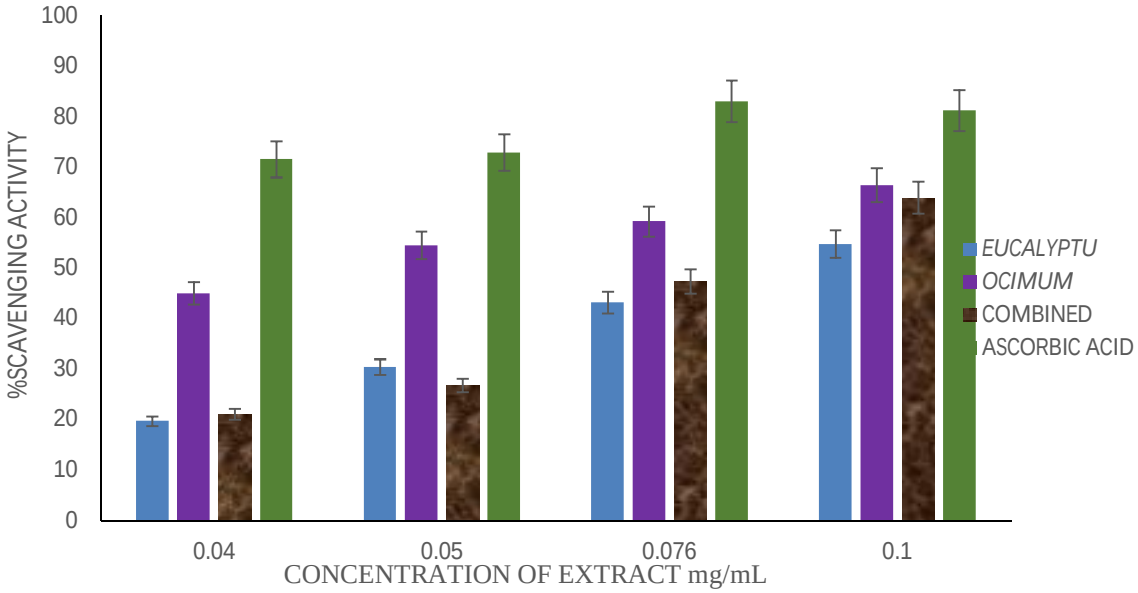


Fig. 4: Free radical scavenging of H2O2 by methanolic leaves extract of Ocimum basilicum, Eucalyptus globulus and combination of the two plants (A)in the afternoon

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B. Antimicrobial Activity

This observed trend of antimicrobial activity is dependent on the following factors; the bacteria strains, the bioactive component of the plants extract, the nature of the interaction between the various plant component, and the time at which the plant leaves were harvested. Generally, the observation made was that, the antimicrobial activity of the methanolic leaf extract of all the samples were concentration dependant. The two bacteria strains used gram positive and gram negative showed varying response to the various plant leaf extract. The highest concentration 1.0 mg/mL of *Eucalyptus globulus* recorded 8 mm as zone of inhibition for early morning extract against the gram negative bacteria *(E. coli*). The gram positive bacteria responded to the combined methanolic leaf extracts of *Ocimumbasilicum* and *Eucalyptus globulus*. The concentrations, 1.0 mg/mL and 0.5 mg/mL recoded 12 and 8mm zone inhibition respectively. The late afternoon sample was active against only the gram positive bacteria recording a zone of inhibition 10 mm. This observed trend was consistent with the work done by Ghaffer*et al.*, (2015). They observed that essential oil of *Eucalyptus* contain 13 components which are all bioactive. Their result was similar to what this study recorded in terms of how active the extract was to the respective bacterial strains. They observed that, the antimicrobial potential of the essential oil was higher against gram positive than the gram negative bacterial. From their result *S. aureus* recoded a maximum zone of inhibition of 31mm.

Zone of inhibition (mm)									
		Morning			Afternoon				
Bacterial	Concentration mg/m L	E	O	C	E	O	C	CTX	CP
E. coli	0.25	-	-	-	-	-	-		
	0.50	-	-	-	-	-	-		
	1.00	8	-	-	-	-	-	31	
S. aureus	0.25	-	-	-	-	-	-		
	0.50	-	-	8	-	-	-		
	1.00	-	-	12	-	-	10		21
C=combination E=*Eucalyptus* O=*Ocimum*CTX=cefotaxime							CP=ciprofloxacin		

Table 1: Zone of inhibition for different plant extract concentration from plants taken at different time of the day

IV. CONCLUSION

The combined methanolic leaves extract of Ocimum basilicum and Eucalyptus globulus has a strong antioxidant activity and it was able to inhibit the growth of S. aureus but showed no inhibition against E. Coli

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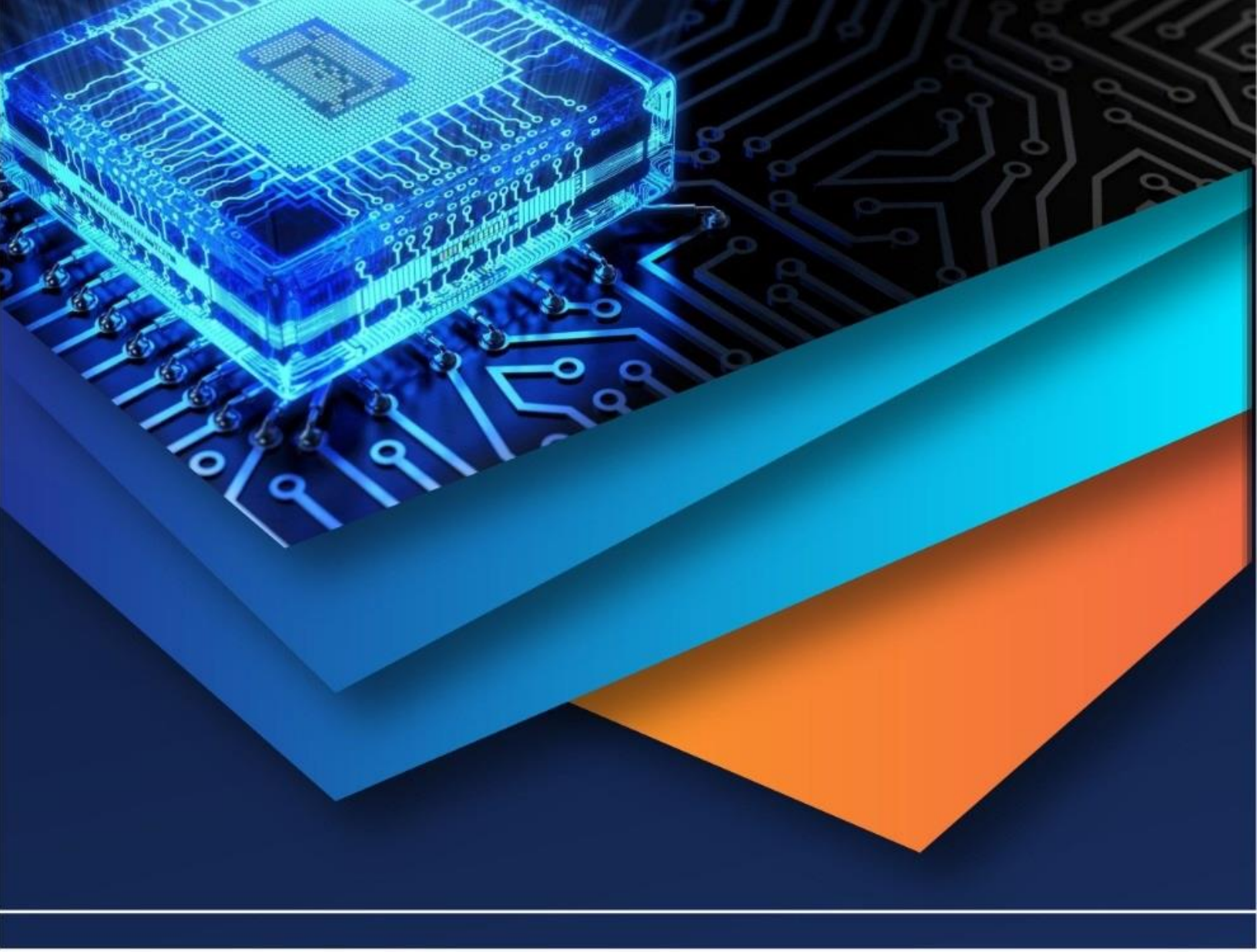
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