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Phytochemical and Antimicrobial activity of various root extracts of Coleus forskohlii Medicinal plant

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Abstract - ***The Phytochemical and antimicrobial activities were studied using ethanol, methanol, chloroform, ethyl acetate, petroleum ether, hexane, hot water, and acetone extracts of Coleus forskohlii, Lamiaceae. Root extracts of Coleus forskohlii were evaluated by agar well diffusion method against six bacterial species and five fungal species. Preliminary phytochemical analysis of the root extract revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, tannins, phenolic compounds and terpenoids in the different root extracts was established. The ethanol extract was more effective against Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae and Staphylococcus aureus, whereas acetone extract was more effective against Micrococcus luteus, Bacillus cereus and Klebsiella pneumoniae. The different extracts were also found to be effective against the test fungi such as Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis, and Cryptococcus neoformans. The results of the present study suggest that Coleus forskohlii roots can be used in treating diseases caused by the tested organisms.***

Keywords - Phytochemical, Antimicrobial activity, root extract, Coleus forskohlii, medicinal plant.

I. INTRODUCTION

There have been enormous scientific reports as the plants are important source of Medicinal compounds to cure various human and animal diseases (Mahesh and Satish, 2008). According to the World Health Organization (WHO), the current survey suggests that many developed countries have a high proportion of the populations, making use of traditional practice of health, especially the use of various parts from medicinally important plants (WHO, 1999). A large number of medicinal plants and their purified constituents have shown that the beneficial therapeutic potentials and exhibits antioxidant activity, anti-diabetic activity etc. Likewise the antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. The ancient indigenous practice of combining and concentrating several plants as decoction (extracting together in boiling water) to treat the whole person and focused different organ system along with the presenting complaint (Scott, 1998). Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Previously *Coleus forskohlii* was also phytochemically analysis by Disticraj and Jayaraman, 2015 and Baskaran, *et al.* 2011)

In an effort to expand the spectrum of antibacterial agents from natural resources, *Coleus forskohlii* belonging to Lamiaceae family (Mint family) has been selected. *Coleus forskohlii* is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma (Kusumoto *et al.*, 1995). It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis (Warrier *et al.*, 1995). It is also known to be a very powerful painkiller, stimulates flow of bile aiding digestion. Certain active principles of this plant are being effective in relieving the intraocular pressure of glaucoma, lowering blood pressure in patients with heart disease, also found to stabilize the cells that release histamine and other inflammatory compounds (Andre *et al.*, 1995). The plant also finds prominent importance in modern medicine. *Coleus forskohlii* has been used historically for menorrhagia in Trinidad (Lam, 2007). In

the present study, phytochemical and antibacterial activity of ethanol and water extract of *Coleus forskohlii* leaves was determined.

II. MATERIAL AND METHODS

A. Plant materials

The *Coleus forskohlii* roots collected during June-July of 2015 in and around Jayantikunj Rewa were authenticated by Department of Botany, Govt. Girls P.G. College, Rewa (M.P.). The voucher specimens were kept in the Environmental Biology Department, A.P.S. University, Rewa (M.P.).

B. Extraction procedure

All the laboratory works are done in Microbiology Deptt. of A.P.S. University, Rewa (M.P.). The plants washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. The roots were powdered and stored in sterile containers for further use. Then these powdered samples (100 g / 100 ml) were mixed in solvents such as hot water, ethanol, methanol, chloroform, Ethyl acetate, Petroleum ether, hexane and acetone extracts for overnight at room temperature. Soxhlet apparatus was used for this extraction (Grouch *et al.*, 1992; Matanjun *et al.*, 2008). The extract from three consecutive soaking were pooled and evaporated under pressure. The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, phenols and furanoids using the method of Harborne (Harborne 1973).

C. Phytochemical test

The extracted samples were stirred with dil Hcl and filtered. This filtrate was tested carefully and used for compound analysis. In this alkaloids (Mayer's test), carbohydrates and glycosides (Molish test), Saponins (Chloroform and H_2SO_4 test), protein and amino acid (Millon's Test), Phytosterols (Libermann- Burchard's test), Phenolic compound (Ferric chloride test) and Tannin (Lead acetate test) were followed (Okigbo and Omodamiro, 2006; Ogueke *et al.*, 2007).

D. Test organisms

The bacterial spp. used for the test were *Staphylococcus aureus (S. aureus*), *Bacillus cereus (B. cereus), Micrococcus luteus,(M.luteus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumonia(K.pneumoniae)* The fungus spp. used for the test were *Aspergillus niger (A.niger), Aspergillus flavus(A.flavus), Candida albicans (C.albicans), Candida tropicalis(C. tropicalis),* and *Cryptococcus neoformans.* All the stock cultures were obtained from Microlabs of A.P.S. University, Rewa (M.P.).

E. Culture media and inoculums preparation

Nutrient agar / broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37°C for 72 hrs and Potato dextrose agar and potato dextrose broth (Himedia, India) were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72 hrs.

F. Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites.

G. Antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Ciprofloxacin (100 µg/mL) *in-vitro* by well diffusion method (Perez, 1999; Bagamboula *et al.*, 2004). Lawn culture was prepared using the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30µl of different solvents with selected *Coleus forskohlii* root extract was added in to the well. The plates were incubated at 37°C for overnight. The activity of the root extract was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents without root extracts were used.

H. Antifungal activity

The extracts were also screened for their antifungal activity in comparison with standard antibiotic ketoconazole (10 µg/mL) in-vitro by well diffusion method (Perez *et al.*, 1999; Bagamboula *et al.*, 2004). Lawn culture was prepared using the test organism on

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Sabouraud's Dextrose agar (SDA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. Using sterilized micropipettes 30µl of different solvents with selected *Coleus forskohlii* root extract was added in to the well. The plates with yeast like fungi were incubated at 37°C for overnight. The plates with mold were incubated at room temperature for 48 hrs. The activity of the root extract was determined by measuring the diameters of zone of inhibition. For each fungal strain, controls were maintained where pure solvents without root extracts were used.

III. RESULTS

The presence of various phytochemicals is shown in Table 1. The results of antibacterial activity are given in the Table 2, which clearly shows that all the extracts have shown antibacterial activity almost equivalent to that of standard against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform, Petroleum ether, hexane, and hot water extracts have shown better activity than the standard against all the six test bacteria. Ethanol extract was more effective against *B.cereus* and *M luteus*. Methanol extract was more effective against *B.cereus* and *S.aureus.* Ethyl acetate extract was more effective against *S.aureus* and *M.luteus.* Acetone extract was more effective against *M.luteus, B.cereus* and *K. pneumoniae.* Chloroform extract was more effective against *S.aureus* and *E.coli.* Petroleum ether extract was more effective against *B.cereus* and *K.pneumoniae* hexane extract was more effective against *S.aureus* and *E.coli.* Hot water extract was more effective against *B.cereus* and *K. pneumoniae*. The results of antifungus activity are given in the Table 2, which clearly show that all the extracts have shown antifungal activity against all the five microorganisms. Ethanol extract was more effective *against C. albicans* and *A. flavus.* Methanol extract was more effective against *A.niger* and *C.neoformans.* Ethyl acetate extract was more effective against *A.niger* and *C.neoformans.* Acetone extract was more effective against *Candida tropicalis, C.neoformans and C.albicans.* Chloroform extract was more effective against *A. niger* and *C.neoformans.* Petroleum ether extract was more effective against *A.niger.* Hexane extract was more effective against *A. flavus* and *C.albicans.* Hot water extract was more effective against *A.flavus* and *C.neoformans.*

Table 1 : Preliminary phytochemical analysis of *Coleus forskohlii* root

Phytochemicals	Test performed	E	M	C	Et	P	H	Aq	Ac
Alkaloids	Dragendorff's test	+	+	-	+	-	-	+	+
Carbohydrates	Molish test	+	+	-	+	-	-	+	+
Saponins	Chloroform and H_2SO_4 test	-	-	+	+	+	+	-	-
Glycosides	Molish test	+	+	-	+	-	-	+	+
Proteins& aminoacids	Millon's Test	+	+	-	-	-	-	+	+
Phytosterol	Libermann-Burchard's Test	-	-	+	-	+	+		-
Phenolic compounds	Ferric chloride test and Lead acetate test	+	+	-	+	-	-	+	+
Flavanoids	Shinoda test	+	+	-	+	-	-	+	+
Terpinoids	Noller's test	+	+	-	-	-	-	+	+
Tannins	Neutral $FeCl_3$	+	+	-	+	-	-	+	+

E – Ethanol; M- Methanol; C- Chloroform; Et – Ethylacetate; P – Petroleum ether; H – Hexane; Aq – Aqueous; Ac – Acetone extracts; (+) Positive (-) Negative.

Table 2 : Antibacterial activity of different root extracts of *Coleus forskohlii* against Differents organisms (Mean±SEM) (mm)

Extracts	Zone of inhibition in mm					
	E.coli	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Staphylococcus aureus*

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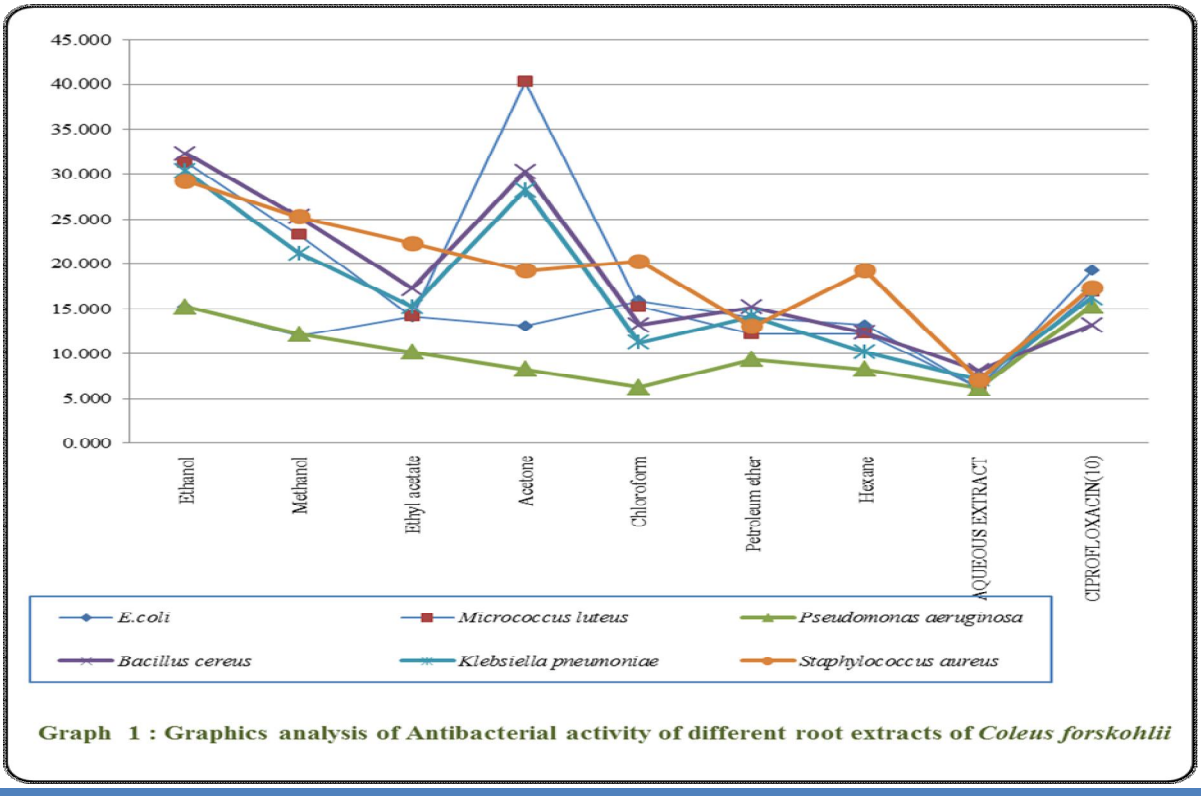
Ethanol	15.231±0.240	31.233±0.251	15.23±0.243	32.232±0.241	30.291	29.231
Methanol	12.023±0.250	23.231±0.242	12.232±0250	25.273±0.250	21.171	25.265
Ethyl acetate	14.13±0.311	14.203±0.260	10.174±0.142	17.231±0.246	15.230	22.270
Acetone	13.11±0.101	40.231±0.253	8.271±0.243	30.23±0.244	28.233	19.231
Chloroform	15.901±0.110	15.23±0.254	6.273±0.250	13.22±0.240	11.270	20.267
Petroleum ether	14.032±0.151	12.231±0.241	9.37±0.321	15.201±0.22	14.201	13.101
Hexane	13.232±0.252	12.26±0.250	8.232±0.251	12.37±0.31	10.173	19.221
AQUEOUS EXTRACT	6.231±0.242	6.242±0.242	6.173±0.151	8.103±0.11	7.170	7.090
CIPROFLOXACIN(10)	19.202±0.257	16.902±0.352	15.37±0.322	13.23±0.240	16.230	17.361

Table 3 : Antifungal activity of different extracts of *Coleus forskohlii* root of against different organisms (Mean±SEM) (mm)

EXTRACTS	Zone of inhibition in mm				
	Aspergillus niger	*Aspergillus flavus*	*Candida albicans*	*Candida tropicalis*	*Cryptococcus neoformans*
Ethanol	10.062±0.121	12.031±0.061	15.073±0.112	10.072±0.121	10.032±0.061
Methanol	17.113±0.101	10.173±0.152	8.071±0.052	8.071±0.101	11.062±0.110
Ethyl Acetate	10.121±0.172	-	5.102±0.101	9.101±0.103	10.101±0.101
Acetone	6.131±0.064	9.013±0.064	10.031±0.051	19.032±0.051	19.053±0.113
Chloroform	11.031±0.061	6.032±0.051	8.031±0.053	7.033±0.052	10.031±0.061
Petroleum Ether	8.062±0.113	5.01±001	6.072±0.101	-	8.022±0.062
Hexane	-	6.033±0.053	6.033±0.043	-	5.031±0.061
Aqueous Extract	5.072±0.121	6.031±0.051	-	-	6.033±0.061
Ketoconazole	10.171±0.151	9.165±0.281	19.701±0.601	28.01±0.501	19.721±0.61



Graph 1 : Graphics analysis of Antibacterial activity of different root extracts of *Coleus forskohlii*

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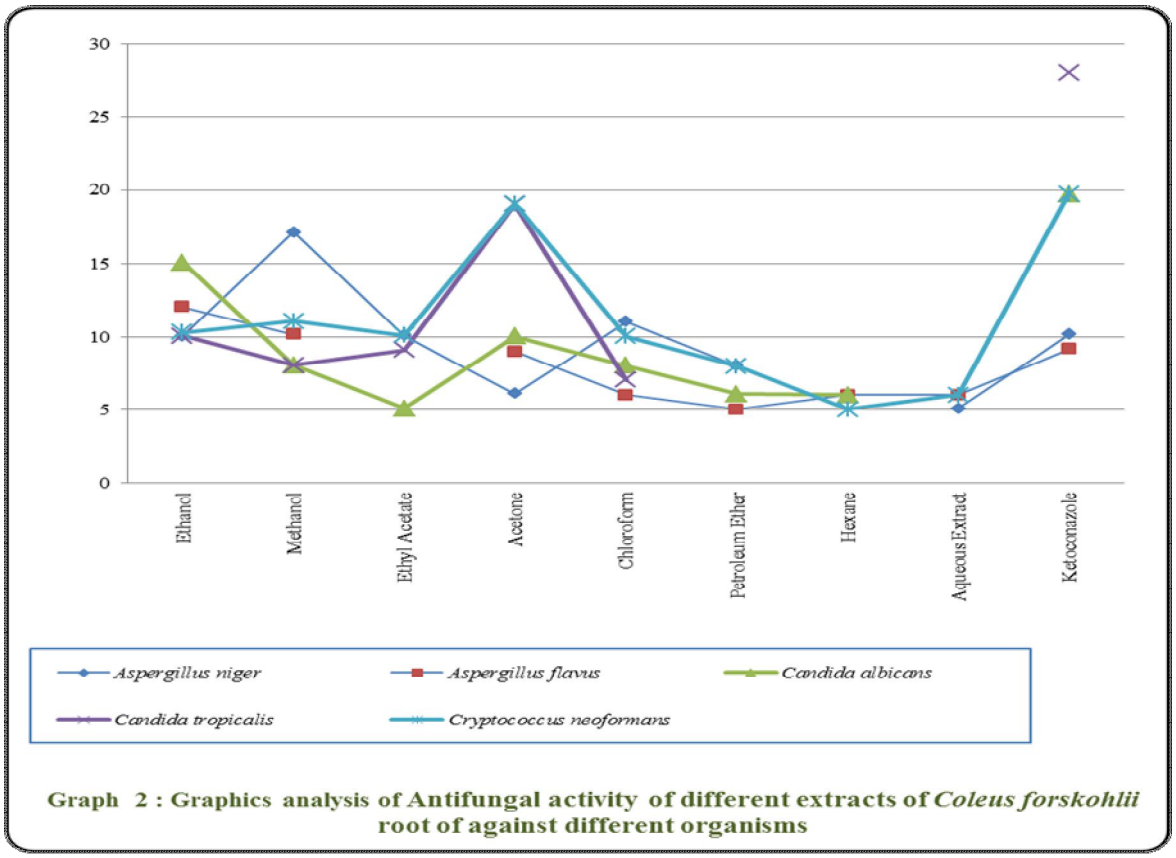

Graph 2 : Graphics analysis of Antifungal activity of different extracts of *Coleus forskohlii* root of against different organisms

IV. DISCUSSION

The therapeutic value of medicinal plants lies in the various chemical constituents in them. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Mohamed, *et al.*, 2010).

Flavonoids are a major group of phenolic compounds reported for their antiviral (Mehrangiz, *et al.*, 2011), antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. The antibacterial activity of the root extracts of *Coleus forskohlii* as recorded in present study may therefore be attributed to the presence of above phytochemicals *i.e* Alkaloids, Carbohydrates, Glycosides, Proteins and aminoacids, Phenolic, Flavinoids, Terpinoids, Tannins in Ethanol extracts and Alkaloids, Carbohydrates, Glycosides, Proteins, Phenolic, Flavinoids, Terpinoids, Tannins in Methanol extract and Saponins, Phytosterol in Chloroform extract and alkaloids, Carbohydrates, Saponins, Glycosides, Phenolic, Flavinoids, Tannins in ethyl acetate extract and Saponins, Phytosterol in Petroleum ether extracts and Saponins and Saponins, Phytosterol in Hexane extracts and Alkaloids, Carbohydrates, Glycosides. Proteins & aminoacids, Phenolic compounds, Flavinoids, Terpinoids, Tannins in aqueous extracts and Alkaloids, Carbohydrates, Glycosides, Proteins and aminoacids, Phenolic compounds, Flavinoids, Terpinoids, Tannins in acetone extracts.

It is concluded that the plant extract possess antimicrobial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto-constituents of herb on the target organism. The antimicrobial activity of the plants may be due to the presence of various active principles in their roots. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs. Based on the results of the present study it is concluded that the *Coleus forskohlii plants* have potent antimicrobial activity against various bacteria and fungi which might be due to the phytochemicals present in the plants. Also, there is further scope to study the identification and purification of active compound(s) involved in this antimicrobial activity of *Coleus forskohlii*.

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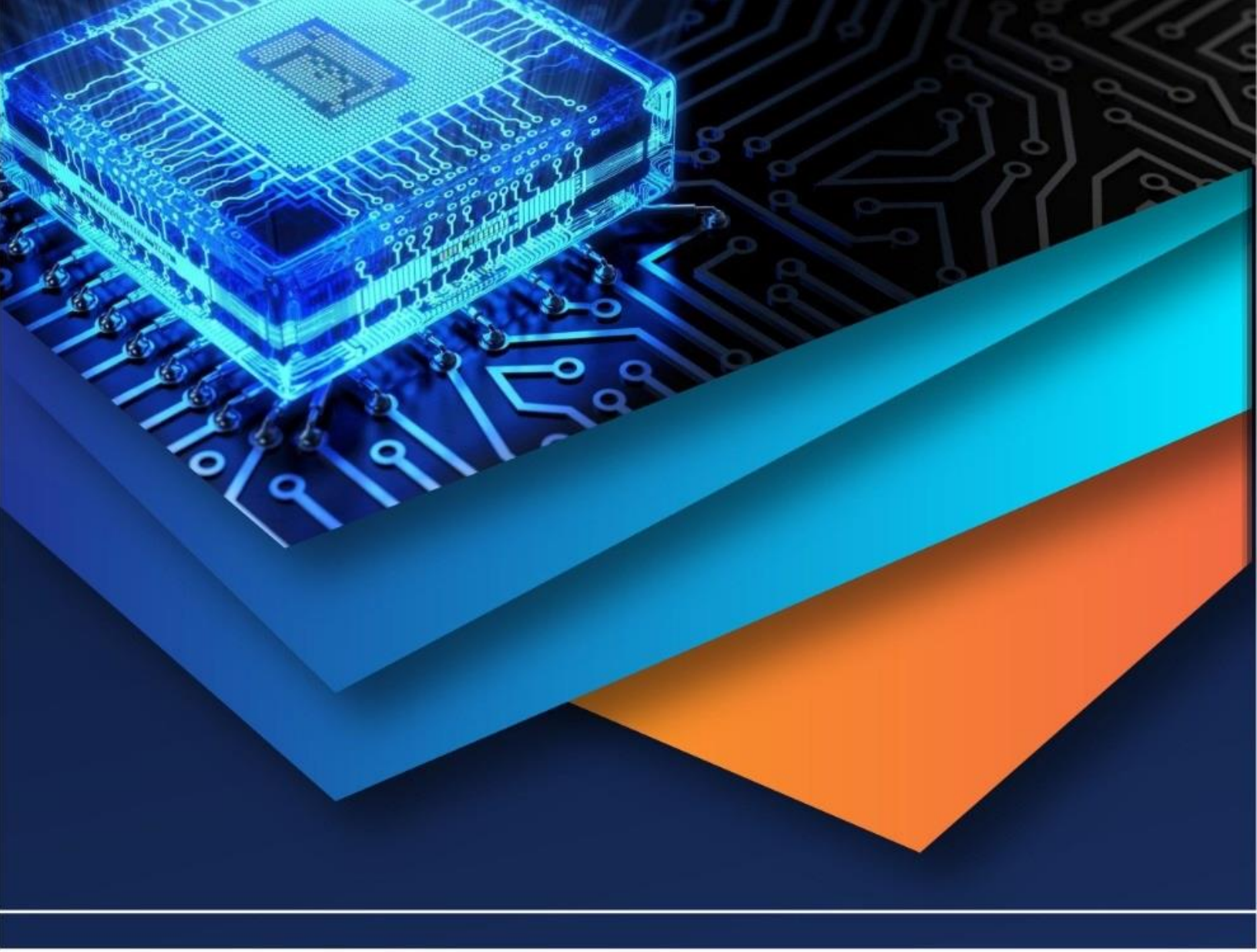

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