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Selection of Phytochemicals from *Calendula Officinalis and Nigella Sativa:* Potent Inhibitors of *Bordetella Pertussis*

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Abstract: ***This paper aims at providing a hypothesis for the treatment of Whooping cough in infants, caused by the pathogenic strain, Bordetella pertussis. The hypothesis is provided by using previously done works against the pathogen worldwide as a reference. Various bioinformatics tools, webservers and software were used in order to complete the task, which include, DrugBank Online | Database for Drug and Drug Target Info, BLAST (uniprot.org), Protein BLAST: search protein databases using a protein query (nih.gov), https://swissmodel.expasy.org/,https://phytochem.nal.usda.gov/phytochem/search, PubChem (nih.gov), RCSB PDB: Homepage, PyRx software and PyMOL. Two drug targets were selected namely, Pertactin and Adenylate cyclase exhibiting 91.96% and 93.88% of the Ramachandran favoured regions respectively. Highest binding affinity was observed to be -11 Kcal/mol, by 12-Ursene-3,6,21-Triol with Adenylate cyclase and -10.8 Kcal/mol by Rutin with Pertactin. According to the data documented and tabulated in the paper and the study conducted concludes that the plant Calendula officinalis and Nigella sativa can be preferred as a treatment option against the whooping cough caused by Bordetella pertussis in the infants and children.***

Keywords: ***Bordetella pertussis, infants, children, whooping coughs, Calendula officinalis, Nigella sativa, Pertactin and Adenylate cyclase***

I. INTRODUCTION

Whooping cough is a highly contagious, vaccine-preventable cough disease that is an important cause of morbidity and mortality worldwide. *Bordetella* is a gram-negative, polymorphic, aerobic coccus. In recent years, the number of cases of whooping cough has increased even in countries where the vaccination rate in early childhood is high. Reasons for whooping cough recurrence include molecular changes in the organism, increased awareness, improved diagnostic skills, reduced vaccine effectiveness, and weakened immunity [1], [2]. The highest morbidity and mortality from pertussis infections are observed in infants who are too young to benefit from immunization. Serious infections that require hospitalization, including intensive care, are predominantly found in children younger than 3 months [3], [4]. As a result, research and public health responses have been directed towards better understanding and containment of the *B. pertussis* epidemic. The standard pathogenesis of the disease in this age group, is categorized by three stages: the catarrhal phase, the paroxysmal phase and the convalescent phase [5]. Clinical remarks, united with results from studies using animal models (BOX 2), recommend that classic pertussis is originated by the adherence of bacteria to the ciliated respiratory epithelium in the nasopharynx and trachea [6], [7]. Adherent bacteria survive innate host defences, such as mucociliary clearance and the action of antimicrobial peptides, reproduce locally and resist abolition by inflammatory cells. Symptoms during this catarrhal phase are similar to those of many upper respiratory infections, such as the common cold. After 1–2 weeks, the illness developments to the paroxysmal phase, which can continue for 1–10 weeks and is considered by periods of normal airway function that are intermingled with multiple severe spasmodic coughing fits, followed by characteristic inspiratory whoops, and often, emesis. The onset of adaptive immunity coincides with bacterial clearance but not with the cessation of symptoms, which typically decline gradually over another month but can persist for much longer (this is known as the convalescent phase) [5]. In infants (<1 year old), pertussis can take a more serious course, in which bacteria disseminate into the lungs and cause necrotizing bronchiolitis, interalveolar haemorrhage and fibrinous oedema [7]. In severe cases, extreme lymphocytosis occurs, which positively correlates with intractable pulmonary hypertension, respiratory failure and death [7]. *Bordetella* species is known to possess adenylate cyclase with a few characteristic properties. It is discharged into the medium by microscopic organisms amid exponential development, and its action is expanded up to 1000-fold by the eukaryotic calcium-binding protein calmodulin [8]. These highlights proposed that this protein may act as a source of virulence in the bacterium. Pertussis toxin, such as pertactin, influence the work of target cells by catalyzing the exchange of ADP ribose from NAD to subunits of one or more guanine nucleotide-binding (G or N) proteins [9]–[13].

This covalent alteration debilitates or impairs flag transduction from particular cell surface receptors to the intracellular arbiter framework. Pertussis poison squares the hydrolysis of phosphatidylinositol, the discharge of arachidonic corrosive, and the incitement of calcium mobilization by a few arbiters of a few cells. The most cell sorts influenced by these toxins are potent effector cells such as neutrophils, monocytes, macrophages, basophils, bone marrow stem cells, and common executioner lymphocytes [8].

II. MATERIALS & METHODS

A. *Choice of Drug Targets Against the Pathogen*

The desired drug targets were selected from the web server (DrugBank Online | Database for Drug and Drug Target Info) [14]–[16] and the FASTA sequence of the specific drug targets were derived from the uniport data base, web server (BLAST (uniprot.org)) by entering the pathogenic strain's name. FASTA sequence was uploaded in the NCBI blast web server (Protein BLAST: search protein databases using a protein query (nih.gov)), to obtain the PDB ID of the specific drug targets.

B. *Homology Model of Drug Target*

Pertactin and Adenylate cyclase are the drug targets chosen against the pathogen i.e., *Bordetella pertussis*. Homology modelling for the above drug targets was performed using Swiss Model Web server (https://swissmodel.expasy.org/). The Data was further authenticated using Ramachandran plot obtained from the web server.

C. *Curation of Phytochemical Database*

Altogether 60 ethnobotanical species of plants were selected and the phytochemical constituents of these plants were derived from various databases, primarily from Dr.Dukes Phytochemical Database (https://phytochem.nal.usda.gov/phytochem/search) which were Downloaded in the form of .sdf files from PubChem database (PubChem (nih.gov)) [17].

D. *Protein-Ligand Docking*

PyRx software was used to accomplish Protein-Ligand docking, using the in-built Auto-Dock Vina software [18]. Minimising the energy of the ligand was done using open babel software followed by minimisation of the protein into a macromolecule [obtained as a PDB file from PDB webserver (RCSB PDB: Homepage)].

E. *Virtual Screening of Phytochemical*

PyMOL was used to visualize the interactions between the Protein-Ligand complex. The interacted structure was visualised in an enhanced manner by highlighting the polar, non-polar and ligand molecules with distinct colours. Picture was saved as a .png format, using the inbuilt option ray in the PyMOL software [19].

III. RESULTS

A. *Drug Targets*

Following are the Ramachandran plots results obtained from Swiss model of the selected drug targets exhibiting their Ramachandran favoured regions numerically and graphically.

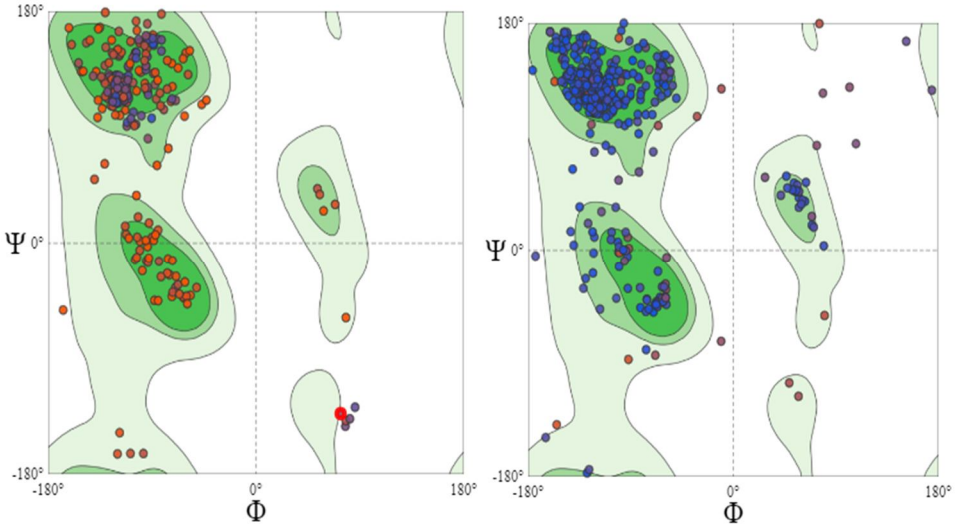

Figure.1: Ramachandran Plots of drug target proteins; A) Adenylate cyclase (93.88%); B) Pertactin (91.96%)

B. Phytochemical Database

A total of 1,102 Phytochemicals were retrieved and saved in .sdf format from PubChem database, that belongs to 60 Ethnobotanical plants. The list of plants included in this study are tabulated in Table.1.

Table.1: List of ethnobotanical plants included in this study.

Papaver somniferum	*Astragalus membranaceus*	*Passiflora edulis*	*Artemisia herba-alba*
v fdsa	*Basella alba*	*Cephalotaxus fortunei*	*Asparagus officinalis*
Dendrobium nobile	*Ocimum gratissimum*	*Dracaena trifasciata*	*Capparis spinosa*
Veratrum viride	*Althea officinalis*	*Petasites japonicus*	*Alpinia galanga*
Anthriscus cerefolium	*Artocarpus altilis*	*Salvia canariensis*	*Lagerstroemia indica*
Eucommia ulmoides	*Nigella sativa*	*Kalanchoe daigremontiana*	*Angelica dahurica*
Portulaca oleracea	*Pyrus communis*	*Daphne genkwa*	*Pueraria pseudohirsuta*
schisandra chinensis	*Origanum onites*	*Angelica sinensis*	*Garcinia cambogia*
Harpagophytum procumbens	*Psidium cattleianum*	*Zantedeschia aethiopica*	*Catharanthus lanceus*
Plantago major	*Raphanus sativus*	*Forsythia suspensa*	*Cyphomandra betacea*
Cyathula officinalis	*Althea officinalis farmacy*	*Satureja hortensis*	*Trigonella foenum graecum*
Vernonia cinerea	*Aegle marmelos*	*Aster tataricus*	*Calendula officinalis*
Myrrhis odorata	*Angelica archangel*	*Agastache foeniculum*	*Ocimum basilicum*
Gleditsia triacanthos	*Bixa orellena*	*Aleurites fordii*	*Alstonia scholaris*
Fritillaria cirrhosa	*Aristolochia debilis*	*Sophora japonica*	*alnus glutinosa*

C. Protein-Ligand Docking

Following are the interpretations obtained as a result from protein ligand docking.

The most significant inhibition potential was demonstrated by 12-Ursene-3,6,21-Triol present in *Calendula officinalis* against the drug target Adenylate cyclase with a binding energy of -11.0 Kcal/mol. However, phytochemical present in *Nigella sativa* i.e., Tannin also demonstrated a significant binding energy of -10.6 Kcal/mol (refer table.2).

The most significant inhibition potential was demonstrated by Rutin which was present in *Calendula officinalis and Nigella sativa*, against the drug target pertactin with a binding energy of -10.8 Kcal/mol (refer table.2).

Based on the docking results, the plant *Calendula officinales and Nigella sativa* has been chosen as the most effective plant tin order to treat and control the targeted pathogen.

Table.2: Summarized docking results of the study

Adenylate cyclase			Pertactin		
Compound	Binding energy (Kcal/ mol)	Plant	Compound	Binding energy (Kcal/ mol)	Plant
12-Ursene-3,6,21-Triol	-11	*Calendula officinalis*	Rutin	-10.8	*Asparagus officinalis, Calendula officinalis, Capparis spinosa, Forsythia suspensa, Nigella sativa, Passiflora edulis, Pyrus Communis, Sophora japonica*

Scutellarin	-10.8	*Plantago major*	Quercetin-3-O-rutinoside	-10.4	*Capparis spinosa*
Tannin	-10.6	*Althaea officinalis, Nigella sativa, Schisandra chinensis*	Tannin	-10.2	*Althaea officinalis, Nigella sativa, Schisandra chinensis*
Rutin	-10.6	*Asparagus officinalis, Calendula officinalis, Capparis spinosa, Forsythia suspensa, Nigella sativa, Passiflora edulis, Pyrus Communis, Sophora japonica*	Hyperoside	-10.2	*Alnus glutinosa, Pyrus Communis*
Eriodictyol-7-O-Beta-D-Glocoside	-10.5	*Ocimum basilicum*	Nicotiflorin	-10.2	*Pyrus Communis, Schisandra chinensis*
Cynaroside	-10.5	*Bixa orellena, Agastache foeniculum*	Isoquercitrin	-10.1	*Althaea officinalis, Artemisia herba-alba, Cyathula officinalis, Nigella sativa, Papaver somniferum, Pyrus Communis,*
Soyasaponin-I	-10.3	*Astragalus membranaceus*	Chrysanthema xanthin	-10	*Calendula officinalis*
Veratrosine	-10.3	*Veratrum viride*	Patuletin-3-rutinoside	-10	*Artemisia herba-alba*
Apigetrin	-10.3	*Plantago major, Bixa orellena, Myrrhis odorata*	Quercetin-diglucoside	-10	*Gleditsia triacanthos*
Quercetin-3-O-Glucoside-7-O-Rhamnoside	-10.2	*Capparis spinosa*	Vicenin-2	-9.9	*Artemisia herba-alba, Calendula officinalis, Trigonella foenum graecum*

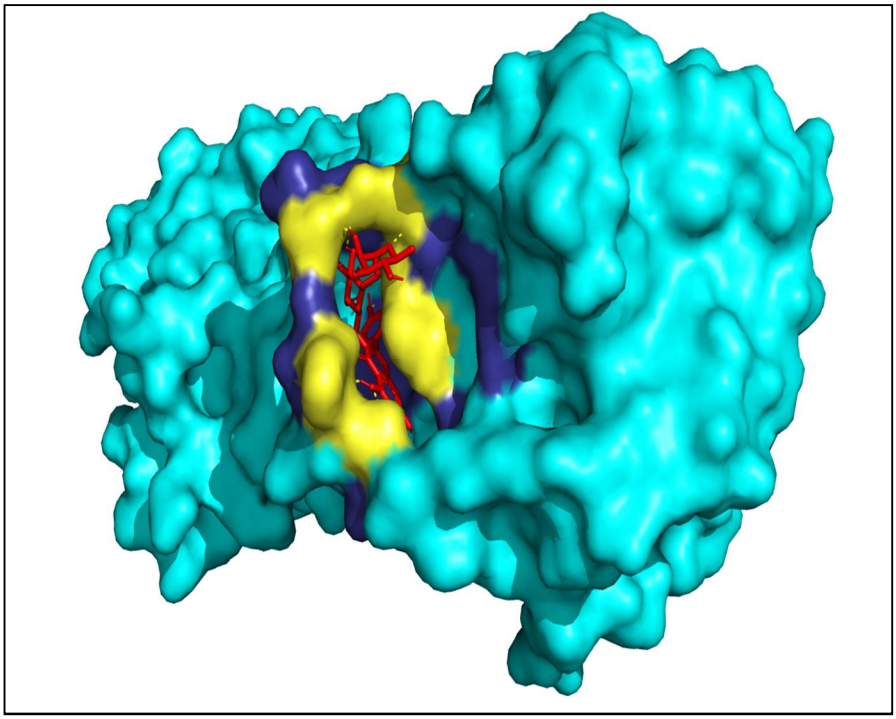

Figure.3: Docking interaction between 12-Ursene-3,6,21-Triol and adenylate cyclase

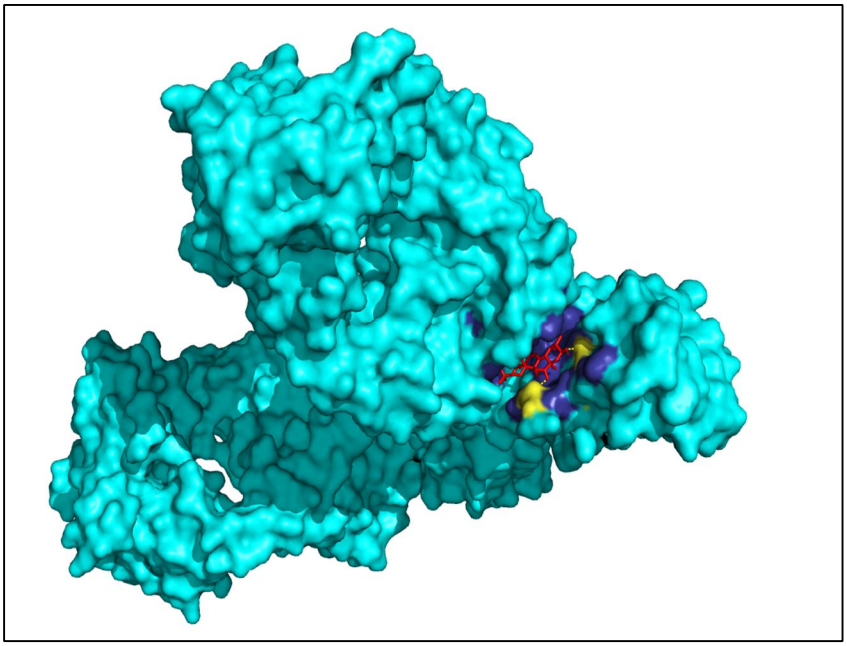

Figure.2: Docking interaction between Rutin and Pertactin

IV. DISCUSSION

Based on the results of docking, the plant *Calendula officinalis* and *nigella sativa* are selected as the most effective plants for treating and controlling this target pathogen due to the highest binding energy offered. These plants contain various potent chemicals in order to attack the multiple protein sites of the pathogen thereby making it inactive or ineffective to produce any infection. The drug targets namely Pertactin and adenylate cyclase are found in the pathogen which are the sites of attack for the phytochemicals present in the plant *Calendula offcinalis* that is 12-Ursene-3,6,21-Triol, Rutin and the plant *Nigella sativa* containing tannin, all of which can act as potent inhibitors of the pathogen's protein structure.

From the previous research articles and documentations, it is found that the plant *Calendula officinalis* and *Nigella sativa* are known to possess a great source of antimicrobial effects against the gram-negative bacteria and hence provides an anticipation for the case studied in this paper. *N. Sativa* has been traditionally used for the remedy of a number of problems, diseases and conditions referring to breathing disorders, digestive tract, kidney and liver function, aerobic vascular damages and immune support system, as well as for wellbeing of an individual [20]. The seeds were historically used in Southeast Asian and the Middle East nations for the treatment of numerous sicknesses and illnesses along with allergies, bronchitis, rheumatism and associated inflammatory diseases. *Nigella sativa* is demonstrated to have antibacterial consequences towards some microorganism like *Staphylococcus aureus, Helicobacter pylori* [20]. Gram-positive bacteria such as *Staphylococcus aureus, S. epidermidis*, other coagulase-negative *Staphylococci*, and *Streptococcus pyogenes* were sensitive towards *N. Sativa* [21]. The Gram-negative bacteria, *Pseudomonas aeruginosa* was sensitive to oil produced by *N. Sativa* seeds [21]. One of the research projects conducted demonstrated that the management of N sativa oil, decreased the pulmonary histopathologic lesion and healthy functional lung cells [22]. The biochemical analysis recognized a rise in antioxidant events, condensed oxidant levels and a decrease in lipid peroxidation and the infiltration of neutrophils-inflammation of the lung tissues dented by hyperoxia [22]. Some studies on calendula officinalis disclosed antibacterial properties in contradiction of *B. subtilits, E. coli*, and *Staph aureus, Trypanosoma brucei* [23], [24]. The antibacterial and antibiofilm actions of water extract of *Calendula officinalis* flowers counter to some of enteropathogenic bacteria was studied [25]. The results showed that the water extract of *C. officinalis* exhibited a good antibacterial activity against all pathogenic bacterial isolates *Salmonella, Shigella dysenteriae, Shigella flexneri, Shigella sonnei and E. coli* [25]. They showed that the extracts of *C. officinalis* leaves were significantly effective against both Gram-positive and especially Gram-negative organisms. High antimicrobial effects of the *C. officinalis* is due to its antimicrobial chemical components [26].

So, from the above discussion it can be concluded that the plant *Calendula officinalis* and *Nigella sativa* can act as the solution against the gram-negative bacteria *Bordetella pertussis* as it possesses great antimicrobial activity contents and has been used previously for the control and treatment of other lungs associated disorder in the humans.

V. CONCLUSION

The results and interpretation of this study concludes that the phytochemicals of the *Calendula officinalis* and *Nigella sativa* plant can be used as a source for developing antibiotics against the infection caused by *Bordetella pertussis* in the infants. As the chemicals obtained will be solely from the plant sources, very less side effects can be expected thus can be used as a better option to eradicate the disease. These advancements and developments in the field of computational biology intermingled with basic life sciences would lead to a better and a safer life for infants and also will help in prevention the spread of the disease in the humans thereby providing betterment to the society.

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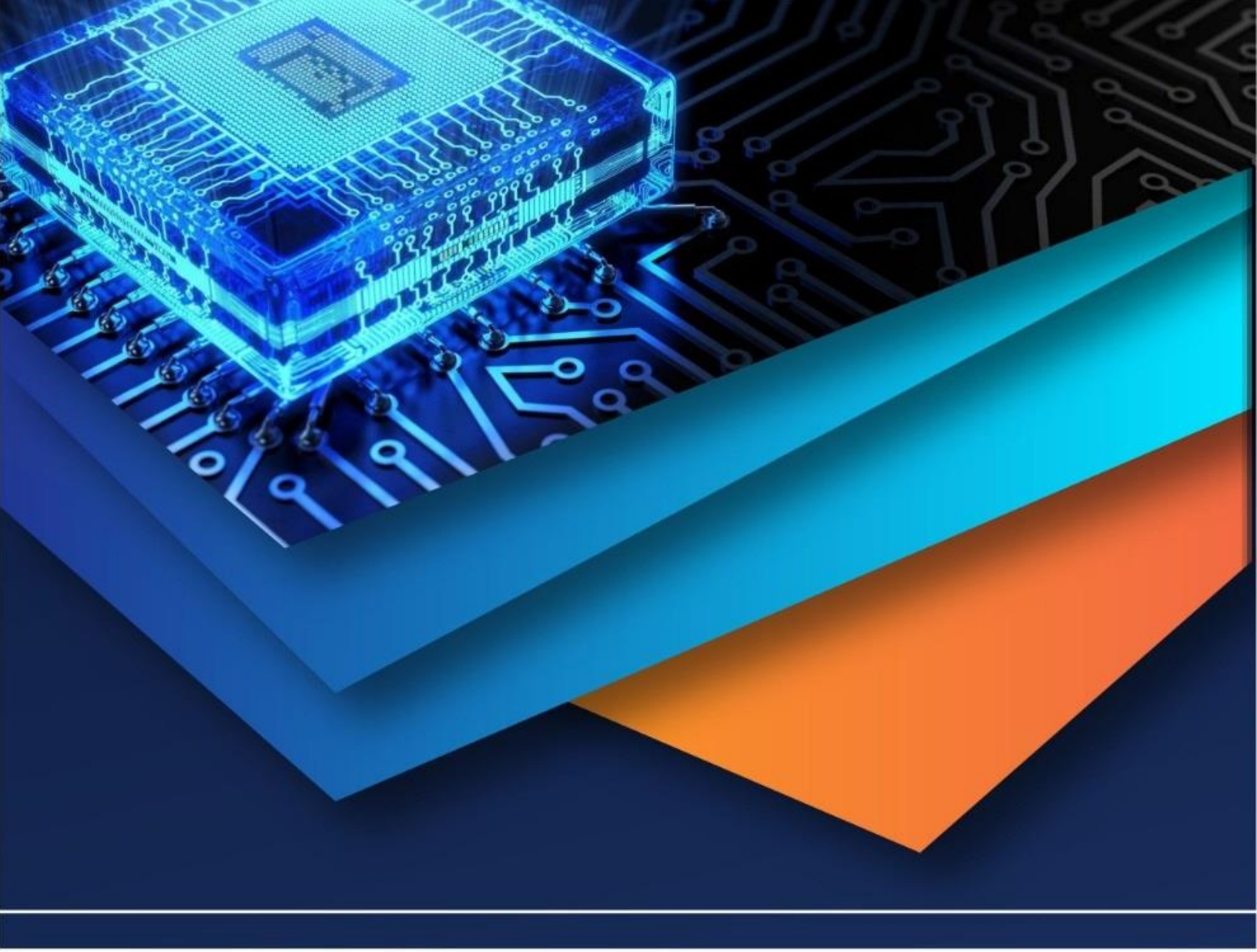

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