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Phytochemical Analysis and Screening of its Antimicrobial Studies of Andrographis Paniculata

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Abstract: ***As aboriginal sources of medications, medicinal plants are used from the ancient times. Andrographis paniculata is one of the highly used potential medicinal plants.A. paniculata generally known as "king of bitters," is an herbaceous plant belonging to family Acanthaceae. This plant is traditionally used for the treatment of common cold, diarrhoea, fever and for jaundice, as a health tonic for the liver and cardiovascular health, and as an antioxidant. It is also used to improve sexual dysfunctions and serve as a contraceptive. All parts of this plant are used to extract the active phytochemicals, but the compositions of phyto constituents widely differ from one part to another and with place, season, and time of harvest.***

The present study was conducted to detect the presence of various phytochemicals present in the methanolic extract of A. paniculata (MAP), both qualitatively and quantitatively. Its antimicrobial potency was also checked using various antimicrobial tests. MAP was tested against various fungal phytopathogens isolated from different spoiled vegetables and fruits and also used against different bacteria isolated from normal human micro flora of skin and teeth.

Though, MAP did not exhibit significant antifungal activity against the phyto pathogens, it showed significant bacteriostatic and bacteriocidic effect against the human resident flora, which often results some opportunistic infections in immune compromised people. However, further study is needed to confirm our results and understand the basis of its antibacterial activity.

Keywords: Andrographis panicualata, Phytochemical analysis, screening of antimicrobial activity, Micro flora from teeth, skin, mouth

I. INTRODUCTION

Medicinal plants are an integral part of human life to combat the sufferings from the dawn of civilization. It is estimated that more than 80,000 of total plant species have been identified and used as medicinal plants around the world. Among these plants, more than 1300 plant species have been used traditionally in Malaysia where the knowledge is being passed down from generation to generation. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various health ailments. Use of the medicinal plants is a core component at primary health care level due to availability, acceptability, compatibility, and affordability. One of such plant species is *Andrographis paniculata* Nees. Used in ancient oriental and ayurvedic medicine.*A. paniculata* commonly known as bhuin neem in odisha. Bhuin neem asthe plant though much smaller in size shows similar appearance and bitter test as that of neem(*Azadirachta indica* L.). It has extremely bitter taste, that's why, it is called as "king of bitter".

It is an annual herb possessing immense therapeutic use. The species is also reported to be a perennial shrub.It is native to India and Sri Lanka. It has been used for centuries in Asia to treat gastro intestinal tract and upper respiratory infection, fever, herpes, throat infection and a variety of other chronic and infectious disease. Indian pharmacopoeia narrates that it is a predominant constituent of at least 26 Ayurvedic formulations. In traditional Chinese medicine (TCM), *A.paniculata*is considered as the herb possessing an important "cold property" useful to treat the heat of body in fever and to dispel toxins from the body.

The most medicinally active phytochemical is and rographolide. *Andrographis paniculata* has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects .Due to its "blood purifying" activity it is recommended for use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers. A primary modern use of *A. paniculata* is for the prevention and treatment of the common cold. It appears to have antithrombotic actions, suggesting a possible benefit in cardiovascular disease. Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like cancer and HIV infections. Most of medicinal plants, even today, are collected from the wild. The continued commercial exploitation of these plants has resulted in receding the population of many species in their natural habitat.

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A. Composition Of Normal Flora

The normal floras of human are exceedingly complex and consist of more than 200 species of bacteria. The makeup of the normal flora may be influenced by various factors, including genetics, age, sex, stress, nutrition and diet of the individual. Three developmental changes in humans, weaning, the eruption of the teeth, and the onset and cessation of ovarian functions, invariably affect the composition of the normal flora in the intestinal tract, the oral cavity, and the vagina, respectively. However, within the limits of these fluctuations, the bacterial flora of humans is sufficiently constant to a give general description of the situation.

B. Composition of Skin flora

Sufficient moisture and abundant food supply facilities growth of microorganism in mouth. However continuous flow of saliva removes microorganism from mouth to stomach and are killed by acidity of stomach.

Desquamation of layer of tissue in mouth is another mechanism that decreases number of microorganism in mouth.

1) Streptococcus *spp*
2) Neisseria *species*
3) lactobacillus
4) Veillonella
5) Actinomycetes

C. Composition Teeth flora

Anaerobic bacteria such as *Bacteroids* and *Fusobacterium* are predominant in teeth. Streptococcus mutans and Streptococcus sanguisare mainly responsible for dental plaque, dental caries and some other bacteria responsible for periodontal disease and gingivitis with beginning of development of tooth, oral flora changes predominantly to gram positive anaerobic bacteria such as

1) Streptococcus
2) Peptostreptococcus
3) Veillonella *spp*
4) Bacteroides

D. Biological details

Kingdom	plantae
Division	Angiosperma
Order	Pensurales
Family	Aeanthaceae
Genus	Andrographis
Species	Paniculata

E. Therapeutic Properties

A. paniculata is prominent in 26 Ayurvedic formulations as evidenced from Indian Pharmacopoeia; while, in Traditional Chinese Medicine it is an important “cold property” herb used to release body heat in fever. The species is well explored therapeutically and effectively used as immunostimulant, and for asthma, gonorrhea, piles, dysentery and dyspepsia, blood purification, Influenza, gastric complaints, diarrhea, pharyngitonsillitis, fever, loss of scalp hair, snakeBite, myocardial ischemia,common cold,diabetes, respiratory tract infections,jaundice18 amongsothers.Thespeciesalsopossessesantiulcerogenic,HIV,antimalarial,antifertility,anti-inflamatory and antihyperglycemicproperties.

F. Habitat

It grows abundantly In southeastern Asia: India (and Sri Lanka) Pakistan and Indonesia but it is Cultivated extensively in China and Thailand, the east and West Indies, and Mauritius. AP is normally grown from seeds ubiquitously in its native areas where it grows in pine, evergreen and deciduous forest areas, and along roads and in villages. In India, it is cultivated during rainy phase of summer season (kharif) crop. Any soil having amount of organic matter Is Suitable for commercial cultivation of this crop. About 400 gm see are sufficient for one hectare. The spacing is maintained 30*15cm.No major insect and disease infestation has been reported. The plants at flowering stage (90-120 days after sowing) are cut at the base leaving 10-15cm stem for plant regeneration. About 50-

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60 days after first harvest, final harvest is performed. In India condition, the yield varies between 2000- 25000 kg dry herb per hectare.

II. REQUIREMENT

A. *Plant Specimen*

Whole plant of *Andrographis panicualata*

B. *Microbial Strains*

Fungal isolates from rotten vegetables and fruits
Bacterial isolates from normal human flora

C. *Growth Media*

Potato Dextrose Agar, Nutrient Broth, Nutrient Agar

D. *Chemicals*

Galic acid, FC reagent, Na_2CO_3, Chloroform, 95% conc. H_2SO_4, $FeCl_3$, 2N HCl, 10% NaOH, Fehling's solution, Mayer's reagent, iodine solution, Isoamyl alcohol.

III. MATERIAL AND METHODS

A. *Collection And Processing Of Plant Material*

The plant were collected from South Bangalore area, near botanical park, Bangalore, Karnataka in the month of February 2019 and authenticated by the botanists of Bangalore City College. The whole plant washed under running tap water to remove dirt. The whole plant dried few days and was crushed into powder and stored in polythene bags for future use. The shade dried plants were powdered

Figure: 1 Plant sample of *Andrographis paniculata*

B. *Andrographis Paniculata Extract Preparation*

A specific amount of powdered plants were subjected to exhaustive soxhlet extraction in methanol (300ml) for 72 hours at 60-70°C. The filtered extract was concentrated and kept in incubator at 37°C for complete solvent evaporation. The crude extract was stored at 4ºC in desiccators for future use. Percent of yield was calculated as follows

Yield % = (w2-w1) /w0*100

Where, w2= the weight of the extract and the container, w1= the weight of the container alone and w0= the weight of the initial dried sample.

The extract was mixed with ethanol and then with distilled water to prepare the required working concentration.

The extract preparation and phytochemical analysis was depicted in flowchart 1.

1) The whole plant of *A. paniculata* was dried and powdered
2) Subjected to suxhlet extraction in methanol
3) Methanolic root extract (MTP)
4) Concentrated & air dried

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5) Phytochemical analysis
6) Qualitative and Quantitative

Figure: 2 Suxhlet Flux

C. Phytochemical Analysis

Qualitative analysis of extract was carried out to determine the presence of various bioactive compounds using the standard qualitative procedure.

1) *Test For Alkaloids:* To 0.5ml of sample and 0.5 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added, presence of green colour or white precipitate indicated the presence of alkaloids.
2) *Test For Trepenoids:* To 0.5 ml of plant sample, 2ml of chloroform was added and then concentrated Sulphuric acid was added gently, formation of red brown colour at the interface indicated the presence of trepenoids.
3) *Test For Phenols:* To 1ml of plant sample and 2ml of distilled water was added then few drops of 10% ferric chloride was added, formation of blue or green colour indicated the presence of phenols.
4) *Test For Tannin:* To 0.5ml of plant sample and 1ml of 5% ferric chloride was added, formation of dark blue or greenish black is indicated the presence of tannin.
5) Test for reducing sugar: To 1ml of plant sample and 1ml of fehling's A, fehling's B was added to the sample, formation of red colour indicate the presence of reducing sugar.
6) *Test For Saponins:* To 0.5ml of plant sample with 1ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise, formation of 1cm layer of foam was indicated the presence of saponins
7) *Test For Proteins:* To 1ml of plants sample were taken and then few drops of HNO3 was added, formation of yellow colour indicates the presence of proteins.
8) *Test For Steroids:* To 0.5ml of plant sample with 4% NaOH solution and few drops of 1% cuSo4 solution were added, violet colour appears indicates the presence of steroids.
9) *Test For Anthocyanin:* 1ml of plant sample mixed with 1ml of chloroform and concentrated H_2SO_4 sidewise, a red colour presence at the lower chloroform layer indicates the presence of anthocynin.
10) *Test For Coumarins:* To 0.5ml of plant sample with 0.5 ml of 10% NaOH was added, formation of the yellow colour indicates the presence of coumarins.
11) *Test For Leucoanthocyanin:* 5ml of isoamyl alcohol was added to 5ml of aqueous sample extract, upper layer appear red in colour which indicates the presence of leucoanthocyanin.
12) *Test For Glycosides:* To 1ml of plant sample with 2ml of chloroform and 10% of ammonia solution was added, formation of pink colour indicates the presence of glycosides.
13) *Test For Carbohydrate:* To 0.5ml of plant sample with 1ml of molisch's reagent and few drops of concentrated sulphuric acid were added, presence of purple colour or reddish colour indicated the presence of carbohydrate.

D. Total Phenolic Compound (TPC)

The total phenolic content of the extract was determined by the folin- ciocalteu (FC) method (Singleton and Rossi, 1965). 200µl of crude extract (1mg/ml), was added to 3.16ml of distilled water, mixed thoroughly with 0.2ml of FC-reagent for 8min, followed by

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the addition of 0.6mlof 10% Na2CO3. The mixture was allowed to stand for a further 60min in the dark and absorbance was measured at 765nm. The TPC was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent for g dry weight.

E. Total Flavonoid Compound (TFC)

The total flavonoid content was estimated by using spectrophotometric method (Quettier et al.2000).The crude extract contained 1ml of the methanol solution of the extract in 1mg/ml concentration and 2% AlCl3 solution dissolved in methanol. The solutions were incubated for an hour at room temperature. The absorbance was obtained at 415nm in triplicate. The same protocol was repeated for the standard solution of rutin and the standard curve was constructed. The content of flavonoid in the extract was expressed in terms of rutin equivalent (mg of RE/g) dry weight of plant material.

F. Bacterial Strain, Maintenance And Storage

Clinical isolates of *E. coli*,*P.aeruginosa*,,*S. aureus*.were procured from Manipal Medical of Bangalore For routine use the culture were maintained on Mueller Hinton agar (MHA) plates. For long term storage, glycerol stocks were prepared by inoculating a single colony into Nutrient broth (NB) incubated at 37°C for 16 hr. To 0.8 ml of this culture 0.2 ml of 50% sterile glycerol was added, mixed thoroughly and stored at 4°C for 1 hr and then stored at -20ºC

G. Growth Medium And Growth Condition

The organisms were cultured in MHA (Himedia, Mumbai) plate or slants for routine use. The medium was prepared according to the manufacturer instruction and sterilized by autoclaving at 15lbs for 20 min. An isolated colony was picked carefully and streaked on fresh agar plate to get isolated colonies. The bacteria were grown overnight at 37ºC in an incubator.

H. Isolation Of Fungal Pathogens

Fungal pathogens were isolated from different rotten vegetables and fruits by inoculating them on potato dextrose agar plates (from a different study).

I. Isolation Of Bacteria From Normal Human Flora

Human skin and teeth were gently rubbed with sterile cotton swabs and then they were gently swabbed on prepared nutrient agar plates by drawing parallel strokes, incubated overnight at 37oC. Selected isolated colonies were streaked on fresh nutrient agar plates by drawing continuous parallel lines. The isolated colonies obtained were used for further study.

J. Antibiotic Sensitivity Test

One isolated colony was inoculated into sterile saline or peptone water. A sterile cotton swab was dipped into the dilute culture medium and swabbed on agar plate. Excess saline was squeezed. Different antibiotic disc (Himedia, Mumbai) were aseptically taken and placed properly on agar plate leaving appreciable gap between two discs. Plates were incubated at 37ºC for 18hr. The clear zone formed around the disc was a measure of the susceptibility of the organism to the antibiotic at a specific concentration

K. Antimicrobial Test

The effect of the different solvent fractions i.e. petroleum benzene, chloroform, acetone and methanol extract of *p.granatum*seed shortly named as HPG,CPG,APG AND MPG against the clinically isolated UTI bacteria (*E. coli*,,*P.aeruginosa S. aureus,*) was determined by disc diffusion, agar well diffusion, modified agar well diffusion method. The cfu/ml was determined in wild and drug treated bacteria by spread plate method

L. Disc Diffusion Method

Discs (5mm diameter) were prepared using whatman filter paper no-1. Different fractions of *p.granatum*seed extracts (HPG,CPG,APG,MPG) of doses 1.5 mg/disc were added on it and kept for some time for complete drying. MHA plates were prepared and swabbed with sterilized cotton bud containing bacterial culture. The drug treated discs were aseptically placed on them. The plates were incubated overnight at 37°C. The clear zones formed around discs were measured.

M. Agar Well Diffusion Method

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A single bacterial colony was suspended in 1 ml of sterilized saline or peptone water, the colony was mixed properly by vortex and incubated at 45°C for 15 min for activation of bacteria. MHA plates were prepared. The bacterial strain was swabbed on the plates with sterilized cotton bud. Then the plate was kept in the incubator for 15 min.

Then wells were dug into it and different fractions of extract (HPG, CPG, APG, MPG- 2.5mg per well) were loaded into the well. The plates were left at room temperature for 1hr for drug diffusion into the media and then incubated overnight at 37°C. The clear zones formed around the wells were measured

IV. RESULT AND DISCUSSION

Andrographis Paniculata whole plant extracts preparation

1) *Yield:* The yield percentage of methanolic extract of andrographis paniculata were 16.59%
2) *Colour:* green colour
3) *Aroma:* bitter smelling
4) *Soluble:* insoluble in oil, Soluble in alcohol, sparingly soluble in water

A. *Phytochemical analysis*

Test name	Results
Trepenoids	+
Alkaloids	+
Tannins	-
Phenol	-
Reducing sugar	-
Saponins	-
Proteins	-
Steroids	+
Coumarin	+
Glycosides	+
Leucoanthocyanin	+
Anthocyanin	-

Table no: 1 phytochemical analysis results

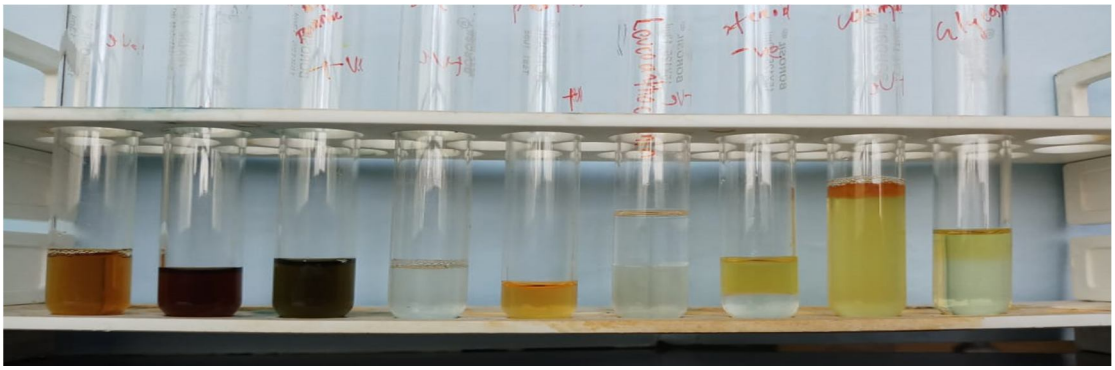

Figure: 3 phytochemical analysis tests

B. *Antibiotic Sensitivity Test*

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Figure: 4 antibiotic sensitivity tests

C. Antimicrobial Test

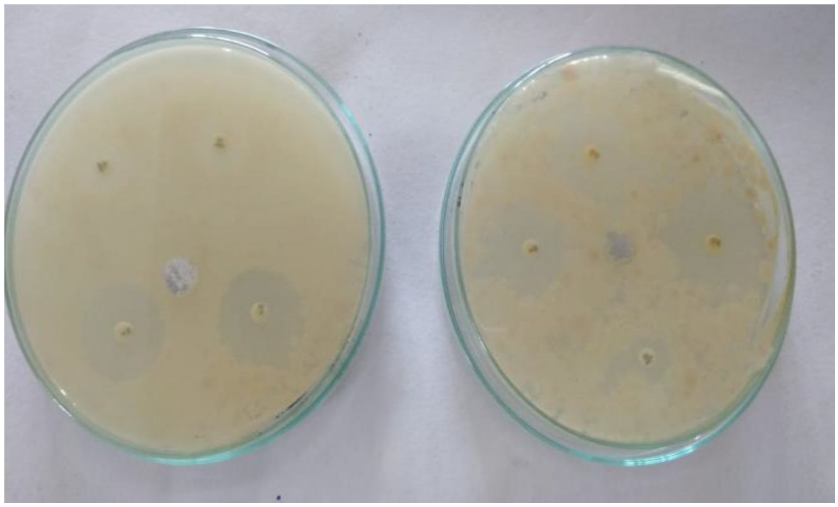

Figure: 5 antimicrobial activity tests

V. CONCLUSION

The experimental studies have shown that methanolic extract of Andrographis Paniculata has anticancer effect by inhibition of nitric oxide synthesis, the phytochemical screening is revealed that extract of Andrographis Paniculata contains alkaloids, flavonoids, trepenes, phenol and tannins, steroids, coumarin, glycosides were present. These studies suggest that these compounds are responsible for the potent antimicrobial effect of plants. Tannis are responsible for the haemostatic and antidiaorrheal properties, saponin act as anti- hyperlipedemic, cardio depressive properties.

The antibiotic sensitivity test was done by taking amplicin, caramycin, ciprofloxacin, oxidase discs, penicillin, chloramphenicol, erythromycin its shown the inhibition zones of particular zone of inhibition which shown the particular organism present on it.

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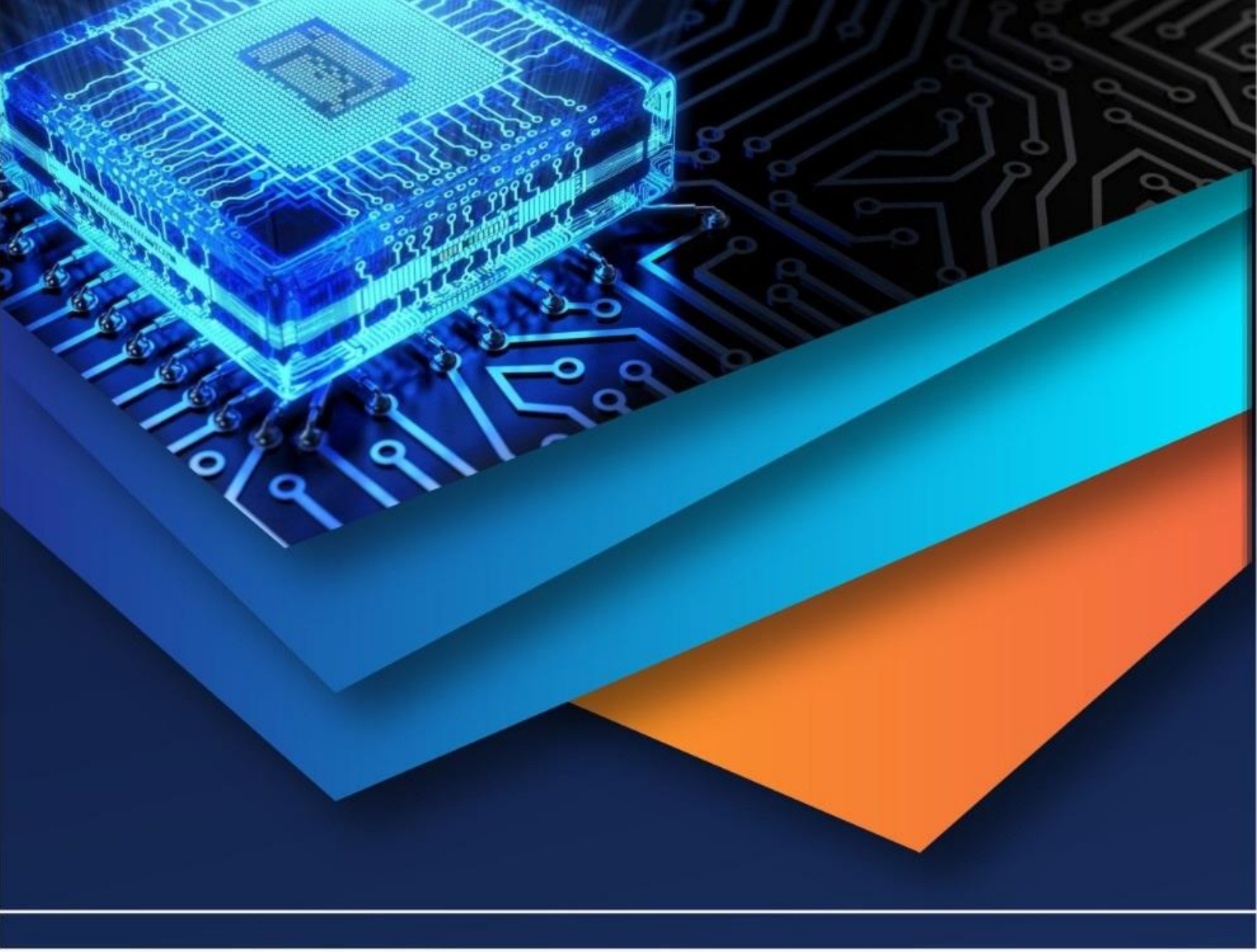

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