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Nutritional Analysis of the Seeds of Holoptelea Integrifolia, Planch.; (Family-Ulmaceae) and Preliminary Phytochemical, Biochemical, Antimicrobial, Antilarvicidal Analysis of Leaf Extract

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Abstract: Medicinal plants are said to have high nutritive value and as a result of that they are prescribed for their therapeutic values. Holoptelea integrifolia (Ulmaceae) is a versatile medicinal plant used in various indigenous system of medicine for curing diseases. Minerals are inorganic compounds needed by our body to regulate chemical reactions and maintain structures. In this present study, antimicrobial activity was done with the leaf extracts. The study revealed that the leaf extracts of Holoptelea integrifolia is showed antimicrobial activity against Staphylococcus aureus and Escherichia coli, Aspergillus niger and Candida albicans. The phytochemical analysis also revealed the presence of Alkaloids, Flavonoids, Saponins, Phenols, Tannins, Glycosides, Anthraquinones, Terpenoids, Carbohydrates and Proteins. Larval mortality of Culex sps was observed after 24 h, 48 h and 72 h of exposure with four high concentrations of ethanolic leaf extract (1 ml, 2 ml, 3 ml and 4 ml) and five low concentrations of crude ethanolic leaf extract (100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm) of the plant. The percentage of mortality decreased with the decrease in the concentration of the test solutions. Minerals analysis of the seeds of Holoptelea integrifolia reveals that the seeds contain phosphorus, potassium, calcium, magnesium, sulphur, copper, iron and zinc.
Keywords: Phytochemical, Antimicrobial, antilarvicidal, nutritional, Holoptelea

I. INTRODUCTION

Medicinal plants contain several bioactive compounds and are of great value for developing some novel therapeutic agents. Due to this tremendous potential they provide new drugs against diseases that influence the health of mankind (Shrinivas, 2008). From the ancient time, most of the medicinal plants are being utilized in various formulations especially in Pakistan and India as Unani medicines and they are also used in Allopathic medicine. Medicinal plants contribute major part in the economic value of India. Phytochemical are biologically active, naturally occurring chemical compounds which are found in plants, which provide medicine for human health further than those attributed to macronutrients and micronutrients (Hasler *et al*., 1999). They protect plant from disease and damage and contribute to the plants colour, aroma and flavour. In general, the secondary metabolites that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al*., 1998 and Mathai, 2000). A compound is a substance made of molecules that contain two or more elements bonded together. A biochemical compound is any compound that contains carbon and is found in living things. They are associated in every process of life-growth, digestion, respiration, etc. In the world, all biochemical compound molecules contain hydrogen and oxygen. They also contain nitrogen, sulphur and phosphorus.

About 1.2 billion people across the world are estimated to suffer from a fungal disease (Vos *et al*., 2012 and Brown *et al*., 2012). They create infections on the skin or mucosa, which responds readily to therapy, but a substantial minority, is invasive and difficult to recognize and treat. An approximate 1.5 to 2 million people die of a fungal infection per year, unparalleled those killed by either malaria or tuberculosis. Four genera of fungi cause fungal diseases. They are: Aspergillus, Candida, Cryptococcus and Pneumocystis. In recent years, antimicrobial derived from the plants are used as synthetic antibiotics that have shown ineffectiveness against several pathogenic organisms, due to increasing drug resistance. Various plant secondary metabolites show antimicrobial activity (Cowan 1999). Plants sources provided a good source of anti-infective agents, which are costly and have lesser side effects. Development of bacterial resistance to the available antibiotics and increasing vogue of traditional medicine has

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led researchers to investigate the antibacterial compounds in plants (Hammaer and Carson, 1999). Japanese encephalitis (JE) is a disease caused by a flavivirus that affects the membranes around the brain. Fatality rate can be as high 60% among those with disease symptoms; 30% of those who survive suffer from lasting damage to the central nervous system. The virus causing Japanese encephalitis is transmitted by mosquitoes belonging to Culexvishuni group (Cx. Tritaeniorhynchus, Cx. Vishuni and Cx. Pseudovishnui) which breed exclusively in flooded rice fields in India and other tropical countries (Hati, 1981).

Sixteen nutrient elements are essential for the growth the reproduction of plants. Nutrients are classified in to two types a) macronutrients and b) micronutrients. Carbohydrates, fats and proteins are usually called macronutrients. Minerals and vitamins are micronutrients. The vitamins and minerals are equally important to our well being although they are needed in very small quantity. Nutrients are substances derived from food during the process of digestion. A nutrient is a chemical that an organism needs to live and grow or a substance used in organism metabolism which must be taken in from seeds (EzeaguIkechukwu, 1996). The source of carbon and oxygen is air, while water is source of hydrogen. Ninety four percent or more of dry plant tissue is made up of C, H and O. Remaining thirteen element, represent less than 6 percent of dry matter, are often divide in three groups (Johnson, 1987). The primary nutrients are nitrogen (N), phosphorus and potassium. Secondary nutrients are sulphur, calcium and magnesium, boron, zinc, copper, molybdenum and chlorine.

II. MATERIALS AND METHODS

A. Study Area (Plate-1 and 2)

Tamilnadu is one of the 29 states in India. Its capital is Chennai, the largest city. Tamilnadu is bordered by the union territory of puducherry and the states of Kerala, Karnataka and Andhra Pradesh. It is situated in the southern most part of the Indian peninsula. Coimbatore is the city in Tamilnadu, South India. The Nirmala College is situated in the district of Coimbatore, which has a salubrious climate due to the presence of forests to the north and the cool winds blowing through the Palakkad gap in the Western Ghats. The College campus is pollution free and eco-friendly and filled with many medicinal trees and has a rich Botanical gardens.

Plate-1: Location Map

Plate-2: Study Area

B. Collection of Samples

The leaves and seeds of selected samples were collected from Nirmala College campus Coimbatore, to find out the qualitative phytochemical and biochemical, quantitative biochemical, antibacterial, antifungal and antilarvicidal activity of leaf extracts were analysed and also seeds of phytochemical and mineral profile were analysed. Leaf sample were collected in the month of January and seeds were collected during the month of April. The data were then processed and represented in tables and charts for further analysis.

C. Plant Authentication

The plant samples were collected from Nirmala College (Coimbatore) identified and authenticated by Botanical Survey of India Coimbatore, Tamil Nadu. The Accession Number - BSI/SRC/5/23/2018/Tech/3386.

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1) *Sample-1*

Systematic position

a) Division : Phanerogams
b) Class : Dicotyledons
c) Order : Urticales
d) Family : Ulmaceae
e) Genus : Holoptelea
f) Species : *H.integrifolia*, Planch.;

Plate-3: Habit of *Holoptelea integrifolia*

Holoptelea integrifolia, Planch.; is a native to Asian-tropical region including India, Nepal, Srilanka, Cambodia, Laos, Myanmar, Vietnam and china. It is a large deciduous tree. It has spreading branches and grows up to 30 to 35m height and 3m girth. Bark is whitish, yellow, grey, covered with blisters, peeling in corky, exfoliate with regular intervals. Leaves are simple, alternate, elliptic ovate, entire glabrous with cordate base, acuminate, nerves 5-8pairs, 5-13cm long and 3.2 to 6.3cm wide. The bark when cut and the leaves when smashed emit an unpleasant smell. Flowers are polygamous greenish yellow to brown in short racemes or fascicles. In bisexual flowers, 5 stamens and in male flowers, 8 stamens are present, basally adnate to tepals, ovary is unilocular and stalked, style very short and its length is 2.5 to 4mm; stigmas 2 in number. The flower appear at the scars of fallen leaves on the tree, from February to March. Fruits are, light brown, obliquely elliptic or orbicular, one seeded samara winged and stalked, indehiscent, 2.5 to 3.5cm long 1.5 to 2.5cm wide. The plant produces a large number of fruits in the month of April-May. It is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism. Bark and leaves are used as better astringent, thermogenic, ant-inflammatory, digestive, carminative, laxative, anti-helmintic, depurative, repulsive urinary astringent and in rheumatism.

2) *Sample-2:* Culex sps.L.; (Mosquito larvae)

Systematic Position

a) Kingdom : Animalia
b) Phylum : Arthropda
c) Class : Insecta
d) Order : Diptera
e) Family : Culicida
f) Genus : *C. sps*, L.;

Plate-4: Closed View of Larvae

Culex sps. L.; is commonly known as the house Mosquito. It is a vector for many diseases, such as Japanese encephalitis, Wile nilevirus, St. Louis encephalitis and also great human nuisance, due to biting. Stagnant water mosquitoes lay egg in clumps, called rafts, of 50 to 300 on the surface of standing water at the edges of lakes and ponds and among the vegetation in swamps and marshes. For nourishment of the larvae of *Culex sps*, the larvae is mainly seen in habitats containing highly polluted water rich in organic matter. The duration of larval stages varies in male and female mosquitoes. The larval stages lasted between 6 and 8 days and pupal stages about 40 hrs (Sajal Bhatacharya, *et al*., 2016).

D. Extraction of Plant Samples

Extraction was done by standard methods. The collected leaves were carefully washed under running tap water followed by sterilized distilled water and were shade dried at room temperature in laboratory for 30-40 days. The dried leaf materials were powdered by using an electric blender and then stored in air tight containers for further analysis. Various organic solvents such as, chloroform, ethanol and water were used for extraction. 40 g of leaf powder is packed with a Whatman filter paper. It is placed into the thimble of a soxhelt apparatus and extracted using chloroform. Appearance of the colourless solvent in the siphon tube was indication of exhaustive extraction and based on that further extraction was terminated. The extract was then transferred into the previously weighed empty petridishes and allows evaporating the solvent. The extract was finally air dried thoroughly to remove all traces of solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an airtight container in a refrigerator below 10°C. After obtaining the chloroform extract and then remove leaf packet from the siphon tube, and it was air dried and again it was extracted using ethanol and water.\

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E. Preliminary Phytochemical And Biochemical Analysis Of Different Extracts

The phytochemical screening of various leaf and oil extracts were analysed by standard methods and shown various phytochemicals constituents such as saponins, phenols, alkaloids, protein/amino acids, tannins, flavonoids, carbohydrates/reducing sugars, anthraquinones, terpenoids and glycosides, (Harbone, 1984 and Wagner *et al*., 1984).

1) Biochemical Analysis done by Anthrone Method and Lowry's Method
2) Larvicidal Bioassay done by Abbott Formula
3) Antifungal Analysis done by Disc Well Diffusion.
4) Antibacterial Analysis done by Disc Well Diffusion.
5) Analysis of Minerals Profile

The minerals like Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Copper, Iron and Zinc were estimated in the standard laboratory by employing Atomic Absorption Spectrophotometer, the results were recorded by following the method of Issac and Johnson (1975).

III. RESULTS AND DISCUSSION

Table-1: Percentage yield of different solvent extracts

S. No	Solvent System	Percentage of yield
1	Chloroform	5
2	Ethanol	10
3	Aqueous	15

HLC –five days running period, 300 ml solvent, 40 g sample
HLE – four days running period, 300 ml solvent, 40 g sample
HLC - seven days running period, 300 ml solvent, 40 g sample

Table- 2: Phytochemical analysis of powdered leaf extracts

S.No	Phytochemical Constituents	Chloroform	Ethanol	Aqueous
1	Alkaloids	-	+++	-
2	Flavonoids	+++	-	-
3	Saponins	-	+	+++
4	Phenols	++	-	+
5	Tannins	+++	+++	+++
6	Glycosides	-	-	-
7	Anthraquinones	-	-	-
8	Terpenoids	+++	+++	-
9	Carbohydrates	++	+	+
10	Protein	+	+	+

The results showed that most of the phytochemicals which are present in the leaf extracts dissolve abundantly in water and polar solvents. Phytochemical analysis, carried out in plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993) Analysis of the extracts showed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. One of the largest and most ubiquitous groups of plant metabolites are the phenolic compounds (Singh *et al*, 2007). Many studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998 and Krings and Berger, 2001). Natural antioxidant mainly derived from plants in the form of phenolic acids, tocopherolsetc (Ali *et al*., 2008). Tannins interfere with protein synthesis and they are bound to proline rich protein. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996).

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Table-3: Qualitative analysis of phytochemical constituents present in the n – hexane extract of the seeds

S. No.	Phytochemical constituents	n-Hexane
1	Alkaloids	-
2	Flavonoids	+
3	Saponins	++
4	Phenols	-
5	Tannins	-
6	Glycosides	-
7	Anthraquinones	-
8	Terpenoids	+++
9	Carbohydrates	-
10	Protein	-

The plant extracts were consist saponins which are known to produce inhibitory effect on inflammation (Just *et al*., 1988). Saponins have the ability of precipitating and coagulating red blood cells. Some of the notable characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sopido *et al.*, 2000 and Okwu, 2004). Potent water-soluble antioxidant and free radical scavenger, flavonoids are also present, which prevent oxidative cell damage and also has strong anticancer activity (Rio *et al*., 1997 and Salah *et al*., 1995). It has the ability to control diabetes induced oxidative stress. Terpenoids are useful in the prevention and treatment of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (Rabi and Bishayee, 2009 and Wagner and Elmadfa, 2003).

Table-4: Carbohydrate and protein contents present in the leaves

Sample	Carbohydrate (mg)	Protein (mg)
HIL	62	27.2

The effect of acute of sulphur dioxide on the metabolism of Holoptelea plants has been analysed. The biochemical changes include deposit of free sugars; especially the reducing sugars in the tissue associated with the accumulation of starch, and enhanced acid phosphate in exposed plants (Farooq M et al., 1985). The result of the final identification on basis of Biochemical analysis is represented in (Table-4).The leaves of Holoptelea integrifolia contains 62mg of carbohydrates and also contains 27.2 mg of proteins.

Table-5: Antibacterial activity of leaf extractsof Holoptelea integrifolia

S. No	Organisms	Zone of Inhibition (mm)			
		Standard Ciprofloxacin (10µg/disc)	Samples (100µg/disc)		
			HLC	HLE	HLA
1	Staphylococcus aureus	42	20	13	10
2	Escherichia coli	40	20	14	08

Different extracts such as chloroform, petroleum ether, methanol and aqueous of stem bark are taken in polarity order. They showed antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa by using disc diffusion method. The vulnerability of the microorganism to the extract of these plants was compared with selected antibiotics ampicillin (standard). Among the various test microorganism used, the chloroform extract of H. integrifolia was found to be very effective. Petroleum ether extract was only effective against E. coli and B. Subtilis: methanol extract was effective against E. coli, aqueous extract against S. aureus, B. subtilis, E.coliand P.aruginosa. Compared with other extracts methanol extract is lesser effective (Paarakh et al., 2011).

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Table-6: Antifungal activity of various leaf extracts of *Holoptelea integrifolia*

S. No	Organisms	Zone of Inhibition(mm)			
		Standard Fluccanazole (10µg/disc)	Samples (100µg/disc)		
			HLC	HLE	HLA
1	Aspergillus niger	12	09	10	08
2	Candida albicans	13	10	10	08

Thus in addition to the microorganisms tested, the extractive solvent was a determinant factor for the extraction of antifungal agents in this study. These results also agree preferably with the suggestions of (Haung *et al*., 2000) that active bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvent used. The aqueous extract showed less antifungal activity as compared to the organic extract possibly because of polarity of antimicrobial compounds that make them more readily extractable in the organic solvent and not suitable quantities of the active compound in the crude aqueous extract (Oloke and Kolawole, 1998).

A. High Concentration

Table-7: Larval mortality rate of ethanolic leaf extracts after 24 hours

Sample	Concentrations	Control	Larval death after 24 hours
HEL	1 ml	0	19
	2 ml	0	20
	3 ml	0	20
	4 ml	0	20

Table-8: Larvicidal activity of ethanolic leaf extracts after 24 hours

Sample	Concentrations			
	1 ml	2 ml	3 ml	4 ml
HEL	95±0	100±0	100±0	100±0

(Mean±Standard deviation)

Table-9: Percentage of Mortality rate

Sample	Concentrations	Percentage of Mortality after 24 hours
HEL	1 ml	95
	2 ml	100
	3 ml	100
	4 ml	100

B. Low Concentrations

Table-10: Larval mortality of crude ethanolic leaf extracts after 72 hours

Sample	Concentrations	Control	Larval death after 72 hours
HEL	100	0	5
	150	0	6
	200	0	7
	250	0	9
	300	0	13

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Table-11: Larvicidal activity of crude ethanolic leaf extracts after 72 hours

Sample	Concentrations				
	100	150	200	250	300
HEL	26.6±2.8	28.3±2.8	36.6±2.8	43.3±2.8	63.3±2.8

(Mean±Standard deviation)

Table-12: Percentage of Mortality rate

Sample	Concentrations	Percentage of Mortality after 72 hours
HEL	100	26
	150	28
	200	36
	250	43
	300	63

Biologically active phytochemicals derived from plant materials can ad as larvicides, pupicides, adulticides, growth inhibitors, repellents and ovipositional deterrents (Venkatachalam and Jebanesan, 2001 and Daniel and Vaiphei, 2005). Control of mosquitoes is mostly directed against larvae and only against adults when necessary. Due to the low mobility of larval mosquitoes, larvae can be effectively controlled. They can be easily identified, where the principal breeding habitats are artificial (lee, 2000). From ecological point of view, insecticides of plant origin are efficient biodegradable and suitable alternative to synthetic products for mosquito control activity. Because they are cheap and easily available, the botanicals have prime importance over synthetic chemicals. Phytochemicals such as alkaloids (Carvalho *et al*., 2003). Steroids (Ghosh et al., 2008; Chowdhury *et al.*, 2008a), phenolics (Tripathi et al., 2001), triterpenes (Rahuman *et al*., 2008), protein (Chowdhury *et al*., 2008b) etc. have good mosquito Larvicidal properties.

Table-13: Mineral profile of seeds

S. No.	Parameter	Results	Daily Value
1	Phosphorus	948.0 mg	1,000 mg
2	Potassium	813.0 mg	3,500 mg
3	Calcium	632.0 mg	1,000 mg
4	Magnesium	108.0 mg	400 mg
5	Sulphur	28.50 mg	14 mg
6	Copper	0.22 mg	2 mg
7	Iron	9.87 mg	18 mg
8	Zinc	3.50 mg	15 mg

Potassium is the macrominerals. It is needed for proper function of blood pressure regulation, carbohydrate metabolism, fluid balance, growth and development, heart function, muscle contraction, nervous system function and also for protein formation. Phosphorus is a major minerals, its function is to maintain acid-base balance, bone formation, energy production and storage and also for hormone activation. Calcium is the one of the most important macrominerals and it required for blood clotting, bone and teeth formation, constriction and relaxation of blood vessels, hormone secretion, muscle contraction and nervous system function. Sulphur is a major macrominerals. Sulphur containing amino acids present in human body. Amino acids are building blocks of proteins. Proteins are macronutrients. Magnesium is necessary for blood pressure regulation, blood sugar regulation, bone formation, energy production, hormone secretion, immune function, muscle contraction, nervous system function and normal heart rhythm and also for protein formation. Iron is a micro minerals and it is required for energy production, growth and development, immune function, red blood cell formation, reproduction and also for wound healing. Zinc is a macrominerals. It is needed for growth and development immune function, nervous system function, protein formation, reproduction, taste and smell and also for wound healing. Copper is a trace minerals and act as a antioxidant. It is required for bone formation, collagen and connective tissue formation, energy production, iron metabolism and also for nervous system function.

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IV. CONCLUSION

The present study provides the useful information about antibacterial properties which are used for the therapeutic purposes. The obtained results provide a support for the use of this plant in traditional medicine and findings of promising source of potential antimicrobials and suggest its further advance investigation. Thus, the plant leaf extracts showed an abundant production of phytochemicals and they can be used in the pharmaceutical industries for producing drugs. Plant can not be suggested for the use in traditional medicine to manage ailments and disorders and also contains biologically active constituents worthy of further investigations for the life of human to be from illness

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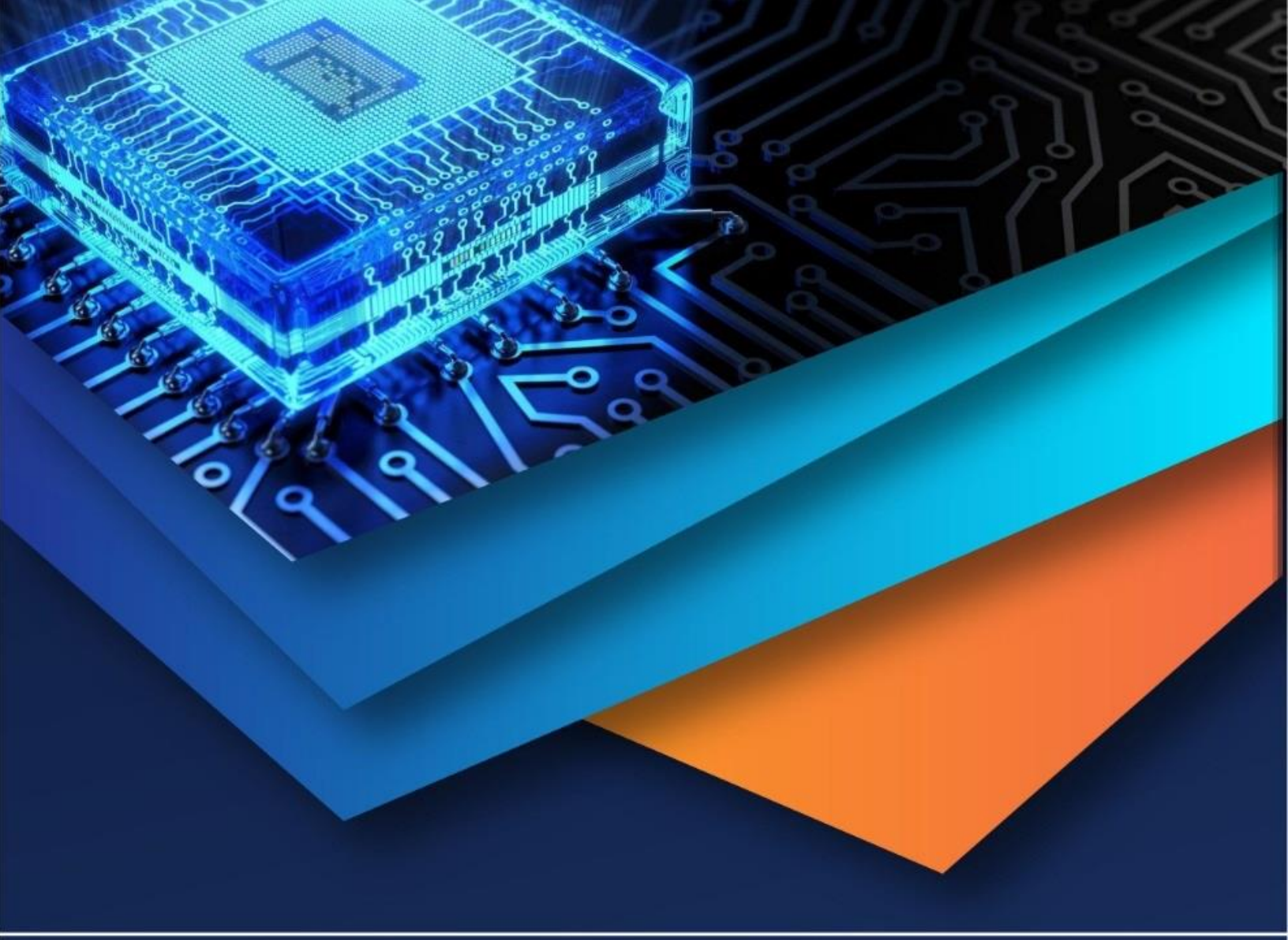

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