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HPLC Analysis of Different Fractions of ETHYL Acetate Leaf Extract of Pterocarpus Santalinus and GCMS Characterization of the Active Fraction with Hypoglycemic Compound

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Abstract: ***The global prevalence of Diabetes is rising at an alarming rate with a reported 1.6 million deaths in 2015.WHO projects that diabetes will be seventh leading cause of death in 2030. The over use of synthetic drugs in diabetes results in higher incidence of adverse drug reaction that motivated the humans to revert to nature for safer herbal medicines Pterocarpussantalinus has been widely used in traditional system of medicine for many ailments. Asanadiganadravya is a group of 23 drugs which is mentioned in Ashtanga Samgraha and Ashtanga Hridya in Ayurveda literature. P.santalinus is one in those 23 drugs .An attempt is made in this study to establish the therapeutic effects through a scientific research. Activity guided fractionation of the leaf extract was carried out by sequential extraction with organic solvents of different polarities. The obtained fractions were subjected to HPLC analysis. The active fraction identified from all the fractions was subjected to GCMS analysis. GCMS analysis of the active fraction showed the presence of compounds that have antidiabetic nature gave an idea in designing new herbal drug that will be presented in the enduring conference.***

I. INTRODUCTION

Diabetes mellitus commonly referred as diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period(1). Diabetes is due to either the pancreas producing less insulin than required by body or the body not responding properly to insulin(2). Diabetes can be classified into three main categories (3).Type1Diabetesmellitus which is due to failure of pancreas to secrete sufficient insulin. This is also called IDDM/juvenile diabetes.Type2diabetes is majorly due to insulin resistance where body cannot respond insulin properly. This is also called as NIDDM/Adult onset diabetes. Gestational diabetes is seen in pregnant women and resolves after baby birth. Of the three types type2 is most common and makes 90% of cases (4).Symptoms of high blood sugar includes frequent urination, increased thirst and increased hunger. Acute complications are diabetic ketoacidosis, nonketotic hyperosmolar coma and death (5). Long term complications include heart disease, stroke, kidney failure, foot ulcers and retinopathy(6) .As of 2015,around 415 million people had diabetes worldwide(7).This indicates 83% of adult population with equal rates in both women and men(8). Various types of oral hypoglycemic drugs are available including insulin which is expensive. So there is a need for herbal drug to treat diabetes without any side effects, low costs and safer for long term use (9). Medicinal activities of plants is due to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins and terpenoids present in these plants (10).World Health Organization reveals that upto 90% of population in developing countries use plants and its products as traditional medicine for primary healthcare (11). The WHO has listed 21,000 plants which are used for medicinal purposes around the world. Among these 2500 species are in India (12). There are about 800 plants which have been reported to have antidiabetic potential (13).Pterocarpussantalinus is one of such plants used for treatment of diabetes (14). Pterocarpussantalinus belongs to the family Fabaceae. Ethno botanical reports shows that P.santalinus is being used to treat diabetes mellitus and related symptoms along with use of other diseases like skin infections, anthelmintic, aphrodisiac, alexiteric, vomiting, thirst, eye diseases, foot ulcers and blood diseases(15).Fruit decoction is used as an astringent tonic to treat chronic dysentery. Wooden cups made of P.santalinus were used to drink water twice a day as treatment of diabetes (16). The ethanolic extract of bark of P.santalinus is proved to have Hypoglycemic activity (17) .The ethno medical use of leaves of p. santalinoides in the treatment of diarrhoea and other gastrointestinal disorders has been scientifically proven (18).Ethanol leaf extract of P. santalinoides and P.santalinus is proved to have hepatoprotective activity in albino rats (19, 20).The leaf extract of P.santalinus has also antibacterial activity(21).In this

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study an attempt has been made to identify the phyto constituents of ethylacetate leaf extract of *P.santalinus* and exposing to Gas Chromatography Mass Spectrum analysis.

II. MATERIALS AND METHODS

A. *Collection of plant material*

Leaves of P.santalinus were collected from the surroundings of Tirupati and Tirumala, A.P.India. The leaves were dried in shade and made into powder using electrical grinder. This leaf powder was stored in airtight container and is used for extraction process.

B. *Preparation of extract and phytochemical analysis*

The dried powder was used for extraction process using soxhlet extractor with ethyl acetate. The extract was then concentrated using Rotary vacuum evaporator and preserved for further analysis. Preliminary phytochemical analysis was performed to the extract and screened for hypoglycemic activity in streptozotocin induced diabetic rats.The extract was identified to have hypoglycemic activity by preliminary screening and that fraction was subjected to bioassay guided fractionation.

C. *Partial purification of leafextract to obtain active fractions*

Ethyl acetate extract was adsorbed on to silica gel by dissolving in pure Methanol. The extract adsorbed silicate was sequentially extracted with different solvent systems ethylacetate, ethylacetate+methanol 50:50, pure ethanol,ethanol+water 50:50 and methanol and all the fractions were collected.

D. *HPLC analysis*

All the fractions were dissolved in HPLC grade methanol (1mg/ml). HPLC model used in the study was LC2010 CHT of Shimadzu company. Sunsil C18 reverse phase column was used for the separation process by gradient elution programmed for pump A(0.1% formic acid pH 3.5-4.5) and pump B(Acetonitrile).The injection volume of each sample was 20µl and the flow rate was 1.0ml/10min.Retention time of each peak was detected by means of UV detector at 254nm.

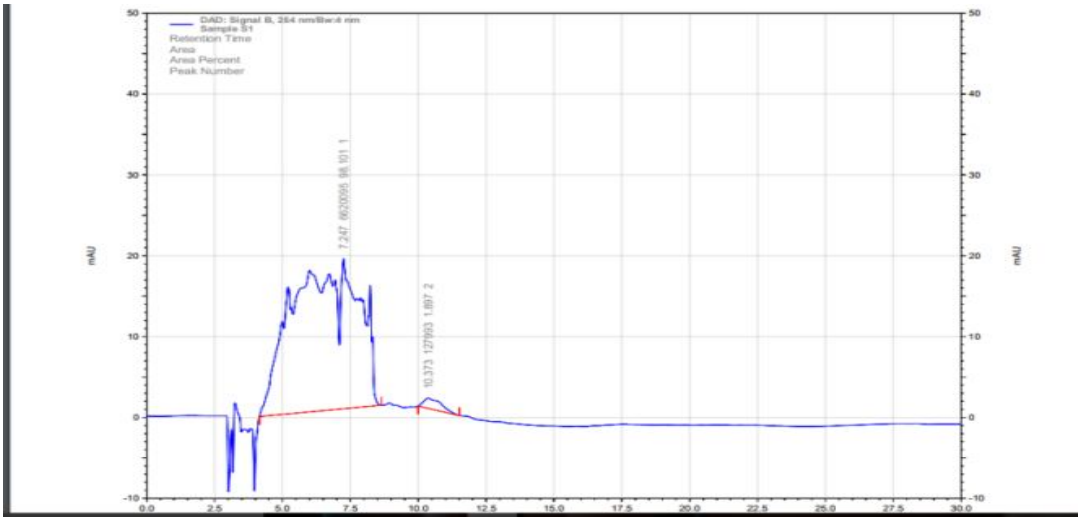
E. *GCMS analysis*

The components of the active fraction identified from HPLC analysis was subjected to GCMS system equipped with HP-5 MS capillary column. Helium was used as carrier gas at a flow of 1.0ml/min. The injection volume is 1 µl. The compounds were identified using NIST and WILEY online libraries.

III. RESULTS AND DISCUSSION

The HPLC analysis of the different fractions is as follows.

A. *HPLC chromatogram of FR-1(pure ethylacetate)*



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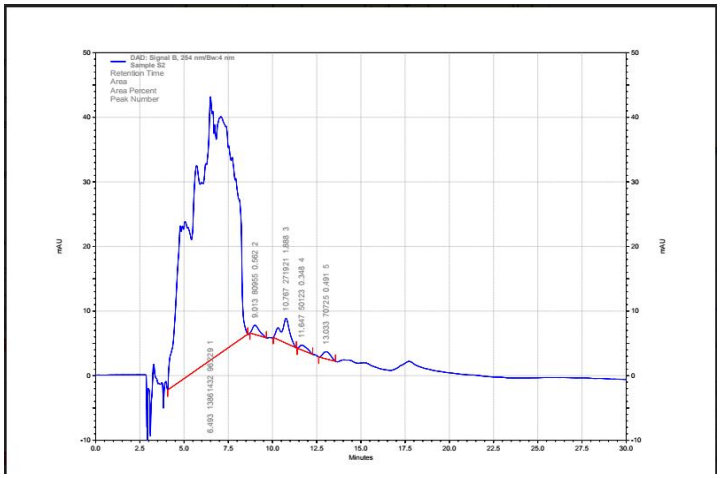
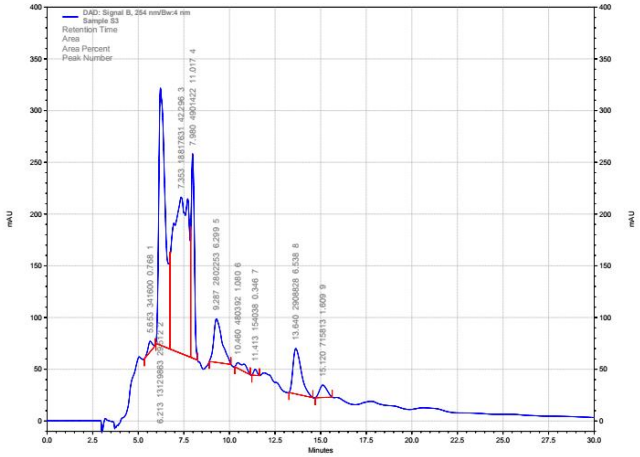
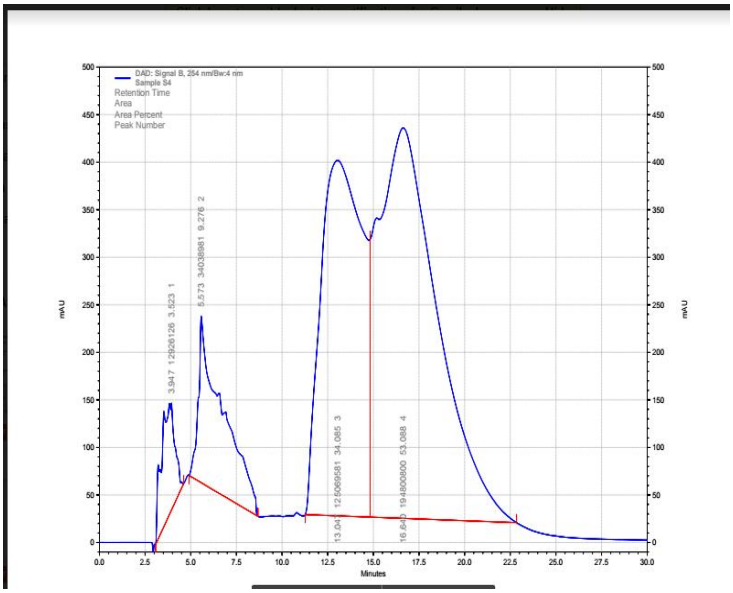
B. HPLC chromatogram of FR-2(Ethylacetate+methanol)



C. HPLC chromatogram of FR-3(pure ethanol)



D.HPLC chromatogram of FR-4(Ethanol and water mixture)



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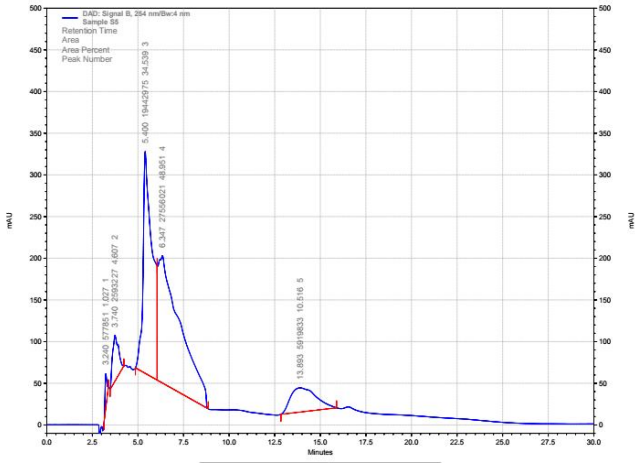
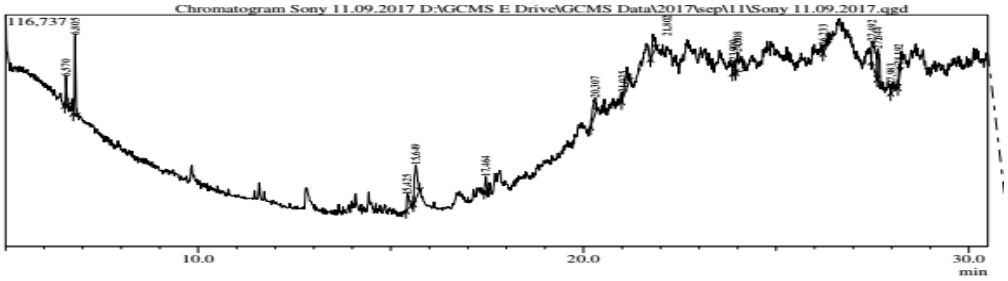
E. HPLC chromatogram of FR-5(pure methanol)



F. GCMS analysis of the active fraction



G. Chemical composition of the active fraction of leaf extract of P.santalinus

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area%	Height%	A/H	Mark	Name
1	6.570	6.542	6.617	3.87	8.63	1.96		PENTANE, 2,2,3,4-TETRAMETHYL-
2	6.805	6.758	6.850	10.89	23.84	2.00	V	1,3-Dioxepane, 2-heptyl-
3	15.425	15.392	15.583	3.68	4.23	3.80		Nonane, 1-iodo-
4	15.649	15.583	15.750	12.56	10.17	5.41		1,2-BENZOLDICARBONSAEURE, DI-(HE
5	17.464	17.425	17.517	3.30	5.07	2.85		1,4-BUTANEDIOL, 2,3-DIETHYL-2,3-DIM
6	20.307	20.183	20.342	7.05	4.96	6.23		4-AMINO-6-METHYL-PIPERIDIN-2-ONE
7	21.025	20.992	21.192	8.70	1.84	20.71		2-Fluoro-6-trifluoromethylbenzoic acid, 4-per
8	21.802	21.742	21.883	6.09	6.09	4.38		4-Methyl-4-nonadecene
9	23.900	23.867	23.950	2.34	3.65	2.80		2-[4-Cyclohexylbutanoylamino]-3-chloro-1,4-
10	24.008	23.950	24.192	10.61	5.90	7.86	V	1,4-DIOXASPIRO[4.5]DECANE, 8-(METH
11	26.233	26.217	26.342	2.48	2.06	5.29		(TRANS-SYN)-BIS[1-PHENYL-4-(1'-PHE
12	27.492	27.467	27.625	13.84	7.05	8.59		4,5-Dihydro-4,4-undecamethylene-2-phenyl-1
13	27.644	27.625	27.717	8.55	9.72	3.85	V	Cholestan-3-one, cyclic 1,2-ethanediyl acetal,
14	27.983	27.967	28.158	3.07	2.41	5.57		Lycorenan, 9,10-dimethoxy-1-methyl-
15	28.192	28.158	28.225	2.98	4.37	2.98		Silane, chlorodiethylheptyloxy-
				100.00	100.00			

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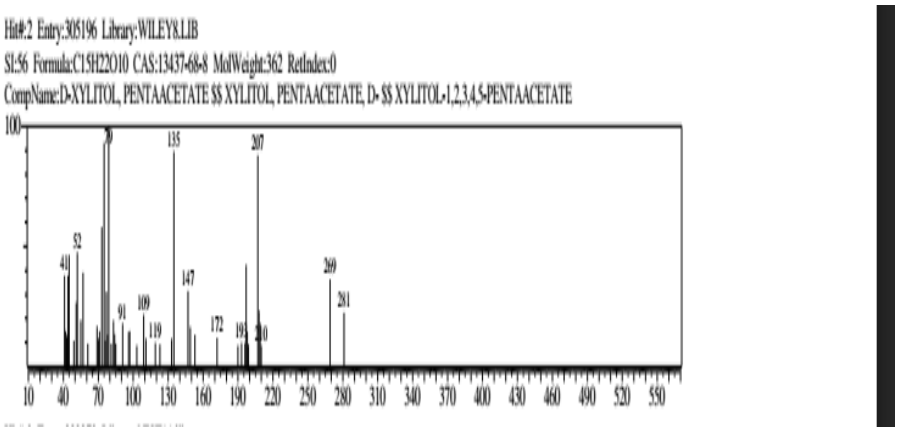

The active fraction was identified by checking for the more number of secondary metabolites contain fraction (pure ethanol fraction containing 9 secondary metabolites) from HPLC analysis and that was subjected to GCMS analysis.

The peaks are marked with retention tim in the GCMS chromatogram of the ethyl acetate extract of the leaves of *P. santalinus*. Their retention time (RT)and the amount of their presence is indicated in table 1.The qualitative analysis showed the presence of 15 compounds. Xylitol is known from literature that it is absorbed slowly in humans. Xylitolis not metabolized and broken down in the stomach like other sweeteners so it reaches parts of the intestines that regular sugar wouldn't. Xylitol contains zero fructose and has negligible effects on blood sugar and insulin. Therefore, none of the harmful effects of sugar apply to xylitol. The glycemic index (a measure of how quickly foods raise blood sugar) is only 7, (22) compared to sucrose which has a glycemic index of 65(23) It can also be considered a weight loss friendly sweetener, since it contains 40% fewer calories than sugar.

Xylitol is a lower-calorie alternative to table sugar. Absorbed more slowly than sugar, it does not contribute to high blood sugar levels or the resulting hyperglycemia caused by insufficient insulin response. This characteristic has also proven beneficial for people suffering from metabolic syndrome, a common disorder that includes insulin resistance, hypertension, hypercholesterolemia, and an increased risk for blood clots(24)Xylitol is used as a sweetener in medicines, chewing gum and pastilles(25)

A study in laboratory rats using an induced model of diabetes found favorable biomarker outcomes for rats given xylitol compared to control rats (26)

IV. CONCLUSION

The GCMS study reveals that active fraction contains a number of phytoconstituents. The presence of D xylitol identified by WILEY online library is known to be used as artificial sweetener. The characteristic feature of xylitol is that it need not require insulin for its metabolism. The further plan of the study is to see the hypoglycemic activity of this compound in cell line cultures.

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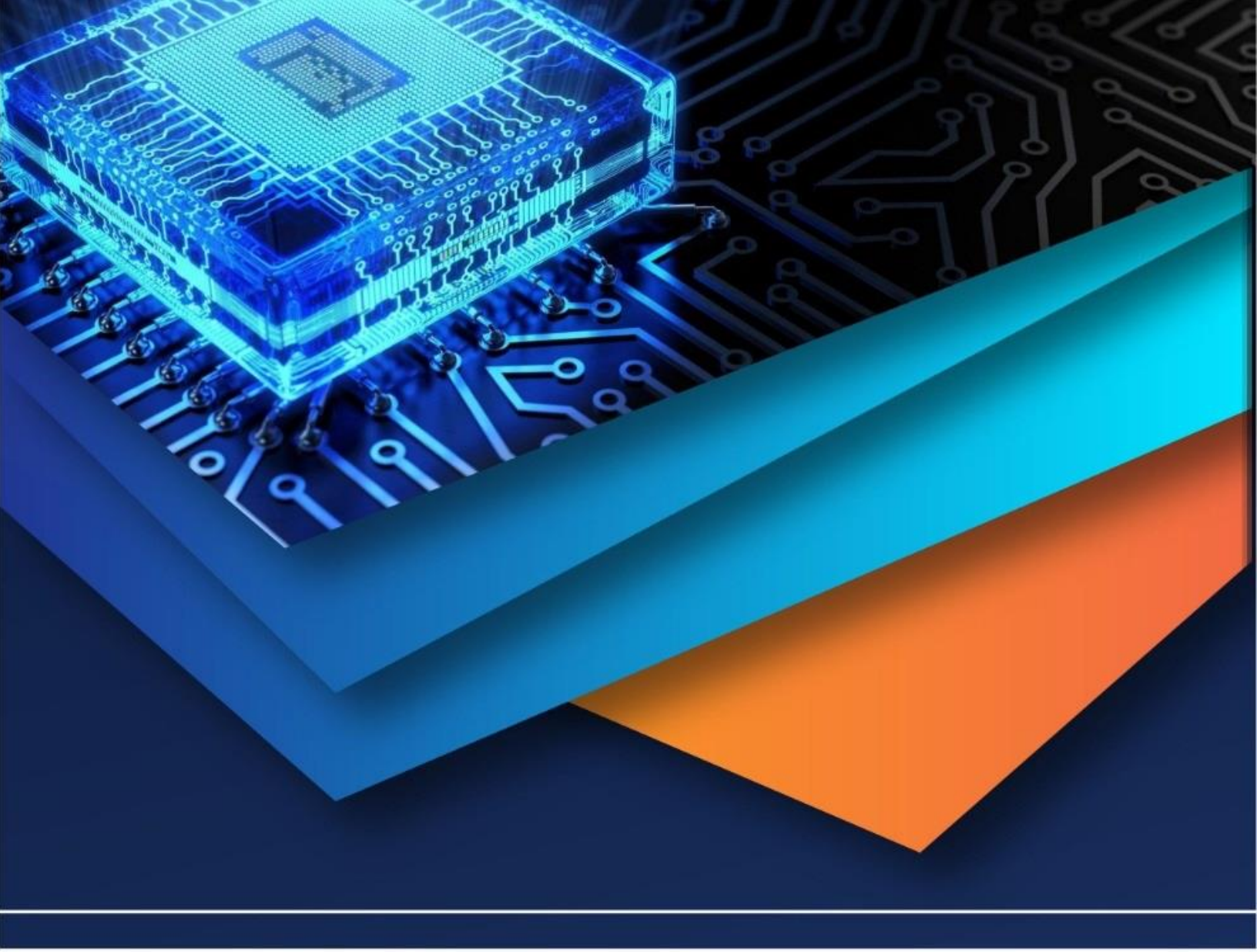

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