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Comparative Study of Phytosterol Content in *Cissus quadrangularis* L. by HPLC

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Abstract: ***Cissus quadrangularis is one of a very important medicinal plant, mostly used for curing up of fractured bones. The plant containes high amount of steroidal components, which give the plant its bone healing property. The present study was an attempt to compare the steroidal contents of Cissus quadrangularis L. plant collected from five districts of Chhattisgarh. The plants were collected and dried and extracted in benzene for TLC. TLC result confirmed the presence of important phytosterols in all the samples of CQ. HPLC analysis was carried out with the methanol extracts of dried samples of all the five selected districts. The result concluded that sample A i.e sample collected from (Ambikapur) Surguja district have higher Stigmasterol content and sample B i.e sample collected from Korba district have higher β sitosterol content than the other samples. The result of the study could be useful for further researches in the field of bone diseases and help in serving society.***
Keywords: Cissus quadrangularis L. HPLC, TLC, Phytosterols, Stigma sterol , β sitosterol

I. INTRODUCTION

Bone diseases are considered to be universal disease (Sharma and Patni, 2007). Number of researches have been carried out in field of bone diseases has always been attempted with different endemic plants and their combinations. *Cissus quadrangularis* is one such plant of India.

Cissus quadrangularis L. is a succulent plant commonly found in tropical countries like India, Srilanka, Malaya, Java and West Bengal. It is a fleshy cactus like plant widely used as a food item in India (Talreja et al.,2017; Jadhav et al., 2013). It is commonly known as Bone setter in English, Asthisamdhani in Sanskrit, and Hadjod in Hindi because of its ability to join bones. The plant has number of medicinal uses in gout, syphilis, piles, diarrhea, dysentery and in kapham (Teware,2013). *Cissus quadrangularis* containes various phytochemicals like steroids, terrpenoides, calcium oxalate, carotene, ascorbic acid etc (Sanayal, 2005;Sharma and Patni, 2006). The steroidal content of *Cissus quadrangularis* play a marked influence on the rate of fracture healing by influencing early regeneration and quicker mineralization of bone callus (Sharma and Patni, 2007).Every part of the plant is used in ayurvedic medicines for solving bone related problems of patients. This study describes the identification and quantification of sterols from the *Cissus quadrangularis* L. samples collected from 5 districts of Chhattisgarh.

II. MATERIAL & METHOD

A. Collection of Samples

Plant material was collected from 5 districts of Chhattisgarh (Ambikapur, Raipur, Korba, Dhamtari and Jagdalpur).

B. Chemicals Required

All the chemicals and solvent system were used are of analytical grade and purchased from Himedia Pvt. Ltd., Mumbai, India.

C. Extract Preparation

Shoots collected were washed thoroughly with tap water followed by distilled water, then wiped and dried under shade followed by oven drying 40-45^{o}C till constant weight is attained. Completely dried shoots were grounded using mixer grinder to obtain a fine powder. Plant powder was refluxed in 30%HCL (v/v) for 4 hours. Each hydrolysed sample was washed with cold water and filtered till ph 7.0 was achieved (Kaul and Staba,1968). Each of the samples then dried at 60^{o}C and then soxlet extracted with benzene for TLC.

For HPLC, plant powder (10gm) was extracted with 50ml of methanol for 12 hrs at 50^{o}C. All the 5 samples were extracted similarly using same solvent. The extracts were filtered through whatmann filter paper no. 1. The filtrate were evaporated to dryness and used for the determination of phytosterols.

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D. Chromatographic Analysis

For TLC, Silica gel (G) coated plates were used, on which all the plant extracts were applied separately along with the standard reference samples i.e. β sitosterol and stigmasterol. The plates were developed in an organic solvent mixture of hexane: acetone (8:2;Fazli and hardman, 1968). Other solvent system such as benzene-ethyl acetate (85:75;Heble at al., 1968),benzene-ethyl acetate(3:1;Kaul and Staba, 1968) were also used but hexane-acetone (8:2)gave better separation of compounds. Such chromatograms were air dried and sprayed with 50%H_2SO_4 (Bennett and Heftmann, 1962) and heated at 100^oC for 10 min until the characteristic colors developed.

E. HPLC analysis

10mg of extracts were reconstituted in HPLC grade methanol and used for estimation of sterols by HPLC.
Standard- β-Sitosteroland Stigmasterol (1mg/mL)
Samples-Test extracts A, B, C, D and E (Methanolic extract, 10mg/mL)

F. HPLC condition

Instrument: Shimadzhu LC- Prominence 20AT
Column: C18 column 250 mm x 4.6 mm, 5µ particle
Mobile Phase: Linear
A: Acetonitrile (94%)
B:0.6% Acetic acid in HPLC grading water (6%)
Flow Rate: 1 ml/min
Injection volume: 10µL.
Absorbance: 209 nm

Sterol Content (mg/g of extract)

$$= (Sample\ Area - Standard\ Area)\ X\ Standard\ injected\ (\mu gms)\ X\ Dilution\ factor$$

HPLC analysis was carried out using 250mm x 4.6mm C18 column. Acetonitrile and 06% acetic acid in Water (94:06) was used as Mobile phase at a flow rate of 1mL/min.

The HPLC chromatogram of standard stigmasterol and β-Sitosterol are presented in Fig. 1 and Fig. 2. Stigmasterol and β-Sitosterol eluted at 3.057 and 3.30 minutes respectively under the standardized HPLC condition. The summary of quantification of stigmasterol and β-Sitosterol content in samples are summarized in Table. 1.

TABLE I
HPLC SUMMARY REPORT

Sl. No	Sample Code	Retention Time (min)	Peak Area	Stigmasterol Content (mg/g of extract)	β-Sitosterol Content (mg/g of extract)	Chromatogram reference
1	Stigmasterol (100 µg/mL)	3.057	406.597			Fig.1
2	β-Sitosterol (100 µg/mL)	3.3	189.568	-		Fig. 2
3	Methanolic Extract of Sample A (10mg/mL Extract)	2.873	81.788	2.01	-	Fig. 3
		3.33	400.623	-	21.13	
4	Methanolic Extract of Sample B(10mg/mL Extract)	2.89	70.739	1.73	-	Fig.4
		3.313	725.231	-	38.25	
5	Methanolic Extract of Sample C (10mg/mL Extract)	3.067	7.857	0.19	-	Fig.5
		3.31	419.006	-	22.10	
6	Methanolic Extract of Sample D (10mg/mL Extract)	2.907	53.464	1.31	-	Fig. 6
		3.327	270.72	-	14.28	
7	Methanolic Extract of Sample E (10mg/mL Extract)	3.07	43.33	1.06	-	Fig. 7
		3.323	474.50	-	25.03	

*Note: Sample A is of Ambikapur (Surguja district), Sample B is of Korba, Sample C is of Raipur, Sample D is of Dhamtari and Sample E is of Jagdalpur (Bastar) district.

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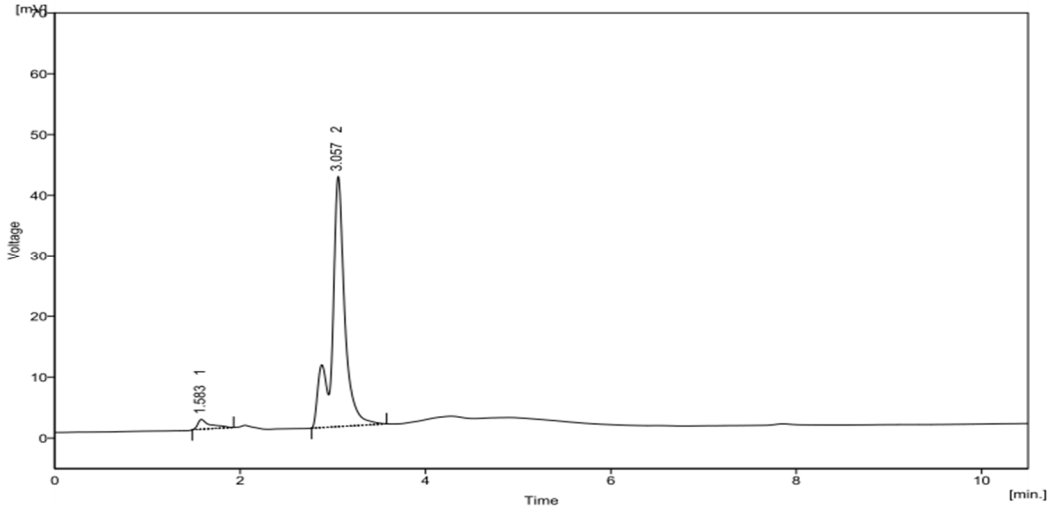

Figure 1: HPLC chromatogram of Stigmasterol (100 µg/mL)

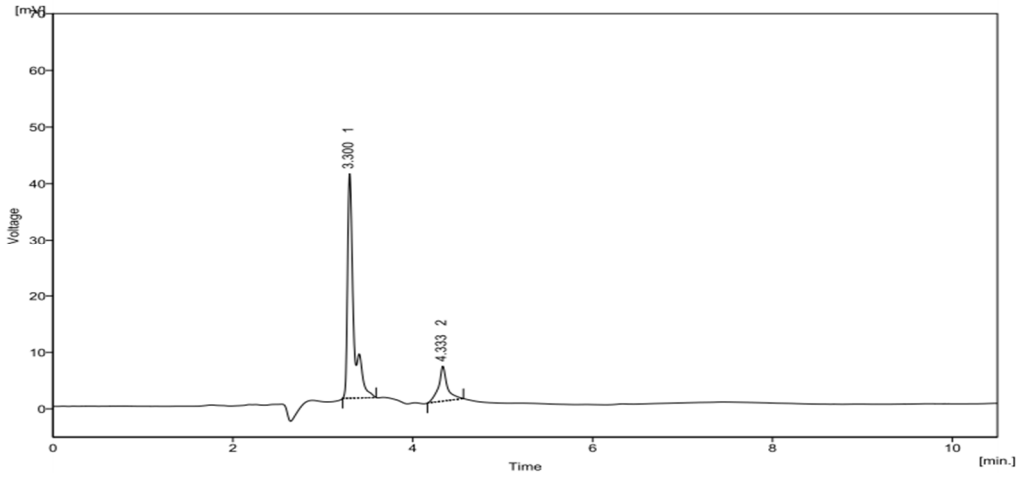

Figure 2: HPLC chromatogram of β-Sitosterol (100µg/mL)

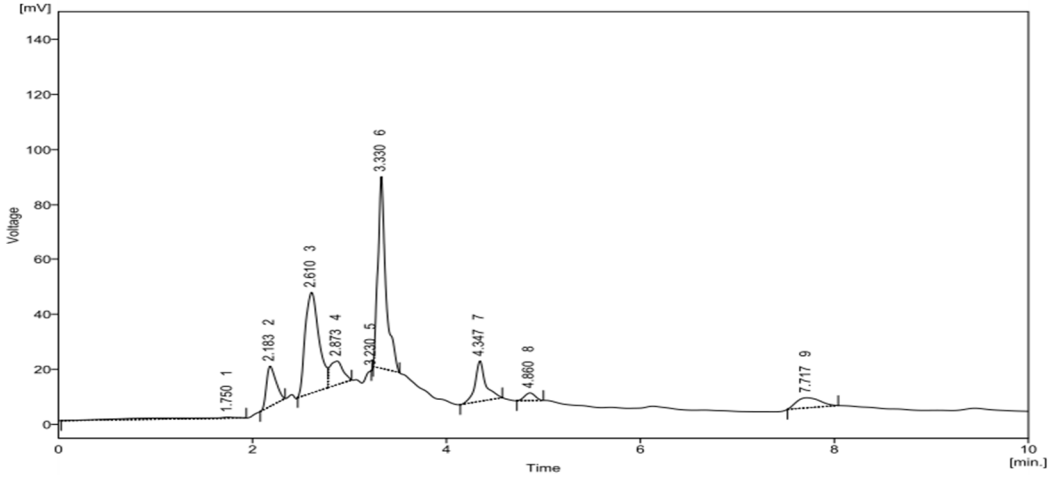

Figure 3: HPLC chromatogram of Sample A (10 mg/mL)

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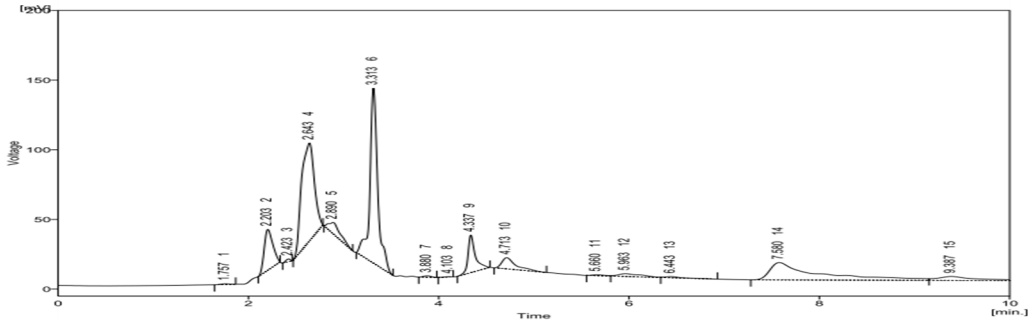

Figure 4: HPLC chromatogram of Sample B (10 mg/mL)

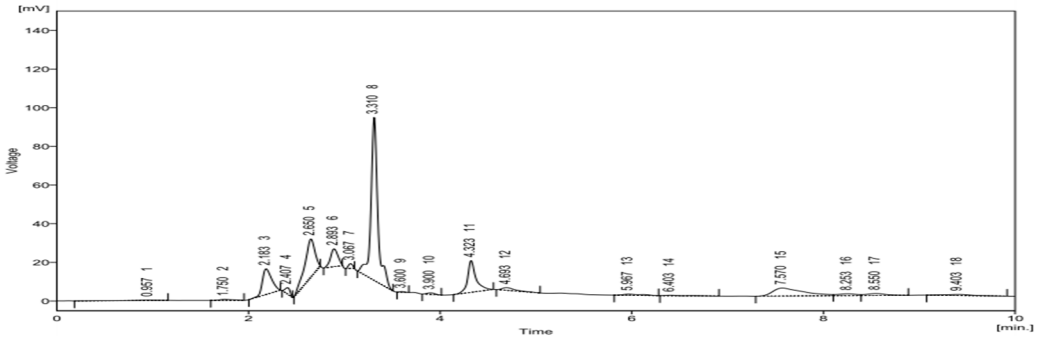

Figure 5: HPLC chromatogram of Sample C (10 mg/mL)

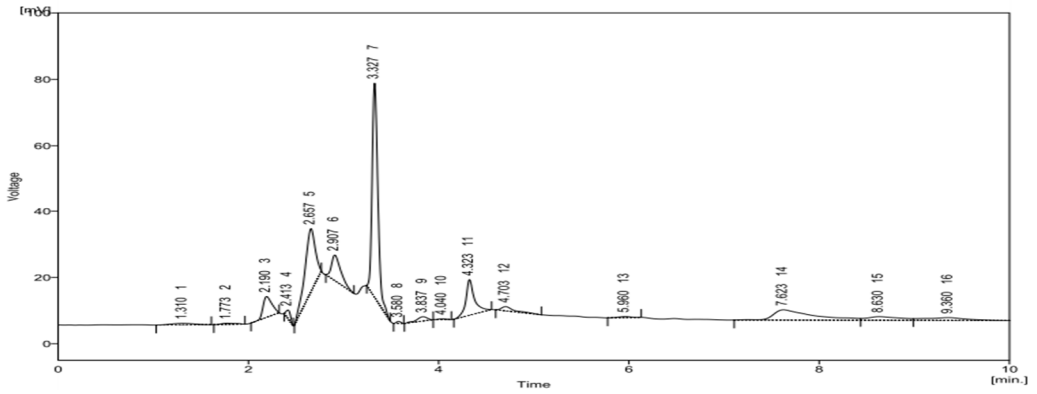

Figure 6: HPLC chromatogram of Sample D (10 mg/mL)

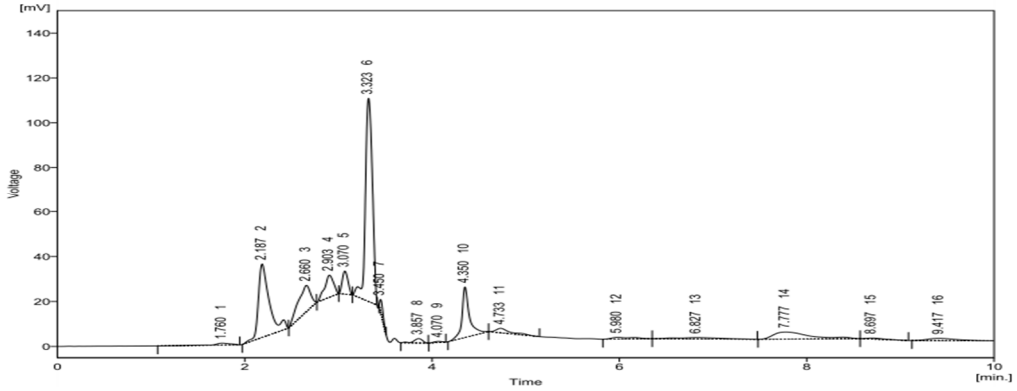

Figure 7: HPLC chromatogram of Sample E (10 mg/mL)

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III. RESULT

TLC analysis of all the 5 extracts of *Cissus quadrangularis* plants collected from five districts of Chhattigarh, showed characteristic spots and Rf value of 0.88 and 0.98 which were comparable to the standards Stigma sterol and β sitosterol respectively.

Stigma sterol and β sitosterol were quantitatively evaluated in all the samples by HPLC. Standard stigmasterol and β sitosterol showed the peaks at 3.06 min retention time and 3.3 min retention time respectively. Fig. 1 and 2 respectively. HPLC of all the five samples i.e A,B,C,D and E showed multiple peaks indicating the presence of several compounds. Sample A , B ,C , D and E showed Stigmasterol and β sitosterol peaks at 2.87 min, 2.89 min, 3.07 min, 2.91 min, 3.07min and 3.33 min, 3.31 min , 3.31 min, 3.33, 3.32 respectively. Fig. 3,4,5,6 and 7 respectively. Sample A showed the higher concentration of stigmasterol i. e 2.01 mg/g wereas sample B showed the higher concentration of β sitosterol i.e 38.25 mg/g.

IV. DISCUSSION & CONCLUSION

The result above showed that Sample A i.e *Cissus quadrangularis* L. collected from Ambikapur, Surguja district of Chhattisgarh and Sample B which was collected from Korba district of Chhattisgarh are rich in the presence of biologically active phytosterols stigma sterol and β sitosterol as compared to the samples collected from other districts. This study is the first report of the comparative quantitative estimation of phytosterols from wild *Cissus quadrangularis* L plants collected from five districts of Chhattisgarh. K. Teware, 2013, reported the HPLC analysis of in-vivo *Cissus quadrangularis* L. plants showed the presence of important flavonoides. Shah, et.al.,2010. reported the development and validation of a reliable RP-HPLC method for the analysis of phytosterols in various extracts of *Cissus quadrangularis* L.Sharma and Patni,2007, described the production, isolation,identification and quantitative estimation ofphytosterols from static cultures of *Cissus quadrangularis* L. The above study describes about the effect of climate and genotypic variations of plants at different regions , because of which the amount of steroidal component varies from region to region. Further the plants with the higher steroidal content can be micropropagated and their content were compared with the wild plants to show the differences in the amount of phytosterols.

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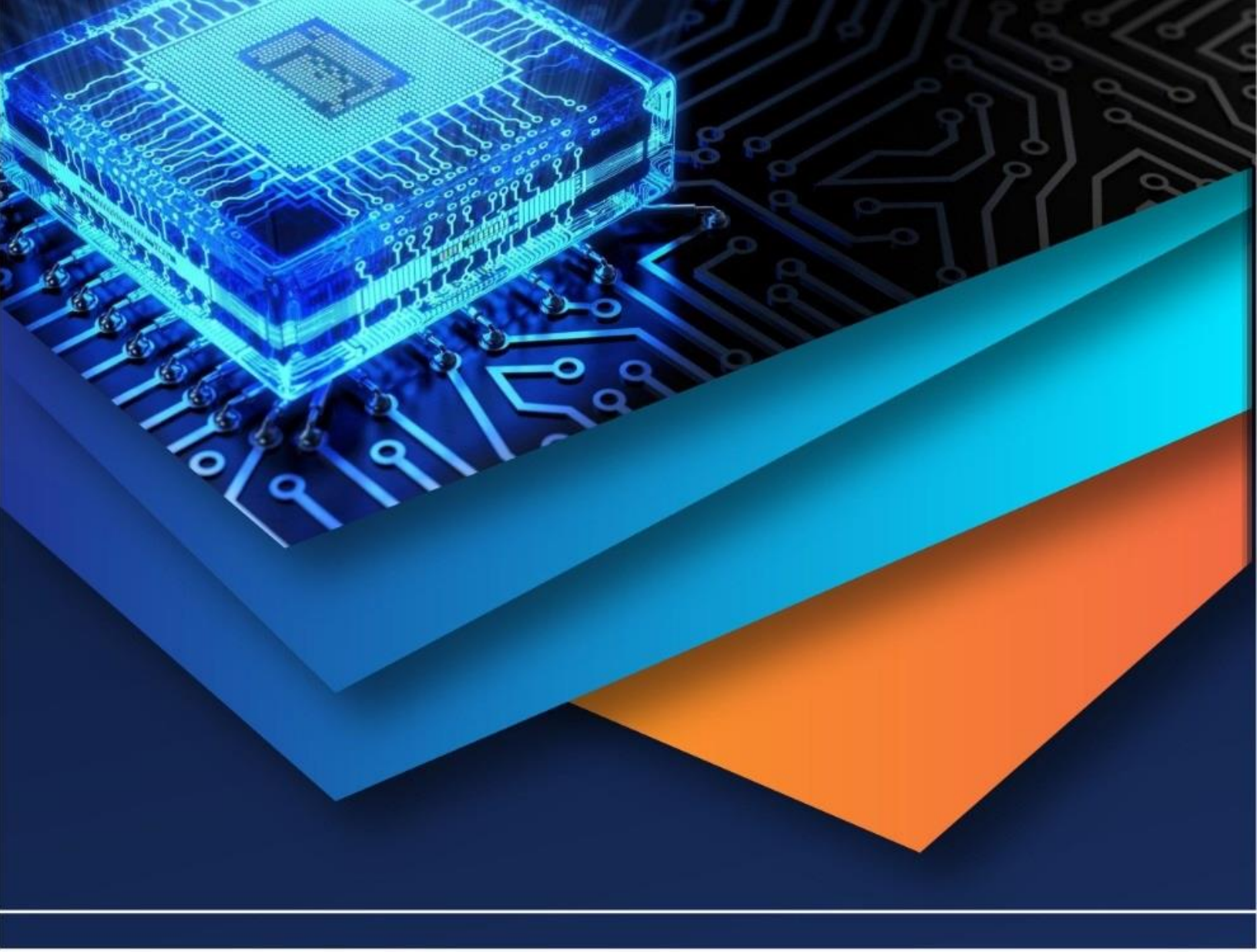

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