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Growth and Characterization of Struvite Crystals by Alpinia Calcarata Stem Extract

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Abstract: ***To investigate the inhibitory effect of methanol extract of Alpinia calcarata stem on the growth of struvite crystals. Struvite crystals were grown by the single diffusion gel growth technique and the inhibitory effect of methanol extracts of Alpinia calcarata stem on the growth of struvite crystals has been studied. The grown crystals were characterized by Fourier Transform Infrared Spectroscopy (FTIR) methods for further confirmations. With an increase in the concentration of methanol extract of Alpinia calcarata stem, the weight of the formed crystals were gradually reduced from 2.83 g to 0.24 g in struvite crystals, respectively. The crystals are harvested from the struvite were characterized by Fourier Transform Infrared Spectroscopy (FTIR) to confirm the functional groups Results obtained are indicated that Alpinia calcarata stem has the potential to inhibit the formation of struvite crystals. This study confirms that using methanol extract of Alpinia calcarata stem can promote the formation of ammonium magnesium phosphate hexahydrate crystals and reduce the nucleation rate of struvite crystals, a major component of triple phosphate urinary stone.***

Key words: Struvite, Alpinia calcarata stem, Fourier Transform Infrared Spectroscopy (FTIR).

I. INTRODUCTION

A large number of people are suffering from urinary stones problems [1]. Urinary stones have been found to contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate or struvite crystals [2-4]. Among the magnesium phosphates, namely,

Ammonium Magnesium Phosphate Hexahydrate (AMPH) commonly known as Struvite and Magnesium Hydrogen Phosphate Trihydrate have also been reported to occur as constituents in renal calculi [5–8] not only in adults but also in children [9, 10]. Struvite calculi, found in 15–20% of urinary calculi [11, 12], are mostly related to urinary tract infections with ureolithic microorganisms in humans and animals [5, 13, 14].

Struvite is also known as triple phosphate stone, infection stone or urase stone. They are found more frequently in women and in persons older than 50 years [15, 16, 17]. Urinary stones are characterized by high recurrence rate therefore requiring a preventive treatment by using the medicinal plants [18, 19].

Alpinia calcarata Roscoe belong to the family Zingiberaceae is a rhizomatous perennial herb, which is commonly used in the traditional medicinal systems [20]. Experimentally, rhizomes are shown to possess antibacterial [21], antifungal [22], anthelmintic, antinociceptive [23], antioxidant [24], aphrodisiac [25], antidiabetic activities, rheumatism, fever and anticancer activity.

It is also widely used to relieve colds and reducing swellings, high blood pressure, diuretic, stomach problems, analgesic, anticandidal, antiplatelet, antispasmodic, antiulcerous hypotensive, insecticidal, muscle relaxant and Uterine stimulant [26,27].

In the present investigation, the effects of methanol extract of Alpinia calcarata stem are used as additives to induce the nucleation and growth of struvite crystals by single diffusion gel growth technique and are reported for the first time.

This study incorporated a multidisciplinary approach in characterizing struvite crystals grown in vitro to help formulate prevention or dissolution strategies in controlling calcium urinary stone growth.

II. MATERIALS AND METHODS

A. Materials and Instruments

Analytical grade of anhydrous methanol, calcium chloride, magnesium acetate, oxalic acid, sodium metasilicate, ammonium dihydrogen phosphate were all purchased from sigma-aldrich, New Delhi, India. Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique.

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B. Collection of Plant Material

The Alpinia calcarata stem were collected in the month of June from the bishop heber college, Trichy, Tamil Nadu, India. The plant was identified and confirmed by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, Tamil Nadu. The voucher specimen number PP008 dated 14.04.2018.

C. Preparation of Methanol Extracts

The Alpinia calcarata stem were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after that it was grinded to a uniform powder of 40 mesh size [6]. The methanol extracts were prepared by soaking 100 g of the dried powder plant materials in 1 L of methanol by using a soxhlet extractor continuously for 10 hr. The extracts were filtered through whatmann filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The obtained residue 1.2 g (stems) was used to prepare the series (0.15, 0.25, 0.50, 0.75 and 1.0%) of methanol supernatant concentrations for in vitro studies (table 1).

D. Growth and Characterization of Struvite Crystals

Glass test tubes were used as a crystallization apparatus and the single diffusion reaction technique was employed [28, 29]. One of the reactants, 0.5 M ammonium dihydrogen phosphate (ADP), was mixed with sodium metasilicate solution the density of 1.04g/cm^3 at pH9.4, so that the pH of the mixture was maintained at 6 and left undisturbed for 2-3 days. After gelation took place, the supernatant solution of 1 M Magnesium acetate was gently poured onto the set gel in various test tubes.

After pouring on each supernatant solution, the test tubes were capped with airtight stopples.

The experiments were conducted at room temperature (37°C). The grown Struvite crystals were characterized using FTIR to verify the compound and structure of the grown crystal. FTIR was performed by Hitachi 570 FT-IR spectrophotometer technique to verify the proper formation of crystal and their purity [30].

Table 1: Supernatant solutions added to the set gels for struvite crystals.

Supernatant Solutions (SS) (Groups and Treatments)	Compositions
I (Control)	10 ml of 1 M magnesium acetate
II (Distilled water)	5 ml of 1 M magnesium acetate +5 ml of distilled water
III (0.15% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.15% of methanol extract of Alpinia calcarata stem separately
IV (0.25% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.25% of methanol extract of Alpinia calcarata stem separately
V(0.50% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.50% of methanol extract of Alpinia calcarata stem separately
VI(0.75% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.75% of methanol extract of Alpinia calcarata stem separately
VII(1.00% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 1.00% of methanol extract of Alpinia calcarata stem separately

E. The Nomenclature Of Different Additive Solution On The Growth Of Chpd Crystals

An attempt was made to study the effect of the methanol extract of Alpinia calcarata stem on the growth of struvite crystals in gel method. The supernatant solutions as given in (table 1) were added to the set gels and the results were noted.

The experiments were repeated four times, to study the effect of the aqueous extract of five medicinal plants on the growth of Struvite crystals, a series of five different concentrations of 0.15, 0.25, 0.50, 0.75 and 1.00% of these each plant extracts were added in equal amounts in supernatant solution and the average weight of the grown crystal were measured.

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F. Statistical Analysis

The masses of the crystals (gm) are presented as the mean ±standard deviation for the control and treatment samples. One-way analysis of variance (ANOVA) followed by tukey's test for multiple comparisons were made between groups. Values of $p<0.05$ was considered to be significant.

III. RESULTS AND DISCUSSIONS

A. Effect of Alpinia Calcarata Stem on Struvite Crystals

The effect of Alpinia calcarata stem on nucleation and crystallization characteristics of struvite crystals is determined by measuring the weight of the formed crystals. In the gel method, the control using pure Mg CH_3COO_2•$4H_2O$ led to the maximum nucleation of crystals growth within 24 h of adding the supernatant solutions Fig. 1 (1a). In the presence of Alpinia calcarata stem, nucleation was delayed and reduced masses of the crystals were observed 96 h after adding the supernatant solutions Fig. 1 (1b-g). Morphology of the harvested crystals after addition of Alpinia calcarata stem as shown in Fig. 2. The largest single struvite crystals having dimensions of 3.2 cm as observed in (Fig. 3a). The sizes of the struvite crystals were reduced from 1.7 cm to 0.2 cm at 1% concentration of extracts was observed in (Fig. 3c-g).With an increase in the concentration of Alpinia calcarata stem from 0.15% to 1% (v/v), the weight of the formed crystals was gradually reduced from 2.83 g to 0.24 g at 5% concentration respectively. The ANOVA statistical analysis was performed and different parameters have been evaluated, and $p<0.05$ has suggested that the correlation is significant as shown in (Table 2).

Recently, growth inhibition studies of Struvite crystals in the presence of some of the herbal extracts [17,18,31] were attempted in literature. In the present work, Struvite crystals growth was reduced due to the inhibitory effect of Alpinia calcarata stem under in vitro conditions. This result indicates that distilled water did not show any inhibitory activity with regard to crystal growth, whereas the methanol extract of Alpinia calcarata stem possessed inhibitory activity due to the presence of bioorganic molecules volatile oil, chiefly sesquiterpene, hydrocarbons, sesquiterpene alcohols, gingerole., starch, tannins flavonoids like galangin [24-27].

Table- 2: ANOVA statistical analysis for harvested Alpinia calcarata stem struvite crystals

CRYSTALS	GROUP	TREATENTS	Mean (gm)±SD
Struvite	A	Control	2.83±0.014
	B	Control+ Distilled water	2.53±0.057
	C	Control+0.15% extracts	$1.47\pm0.014^{a,b}$
	D	Control+0.25% extracts	$0.68\pm0.014^{a,b,c}$
	E	Control+0.50% extracts	$0.41\pm0.014^{a,b,c,d}$
	F	Control+0.75% extracts	$0.38\pm0.014^{a,b,c,d,e}$
	G	Control+1.00% extracts	$0.24\pm0.014^{a,b,c,d,e,f\text{-ns}}$

Values represent mean (gm) ± S.D (n=4) Comparisons between means are as follows. a: A vs B-G, b: B vs C-G, c: C vs D-G, d: D vs E-G, e: E vs F-G, f: F vs G. Statistical significance were considered to be $^{a}p<0.05$, $^{b}p<0.05$, $^{c}p<0.05$, $^{d}p<0.05$, $^{e}p<0.05$.

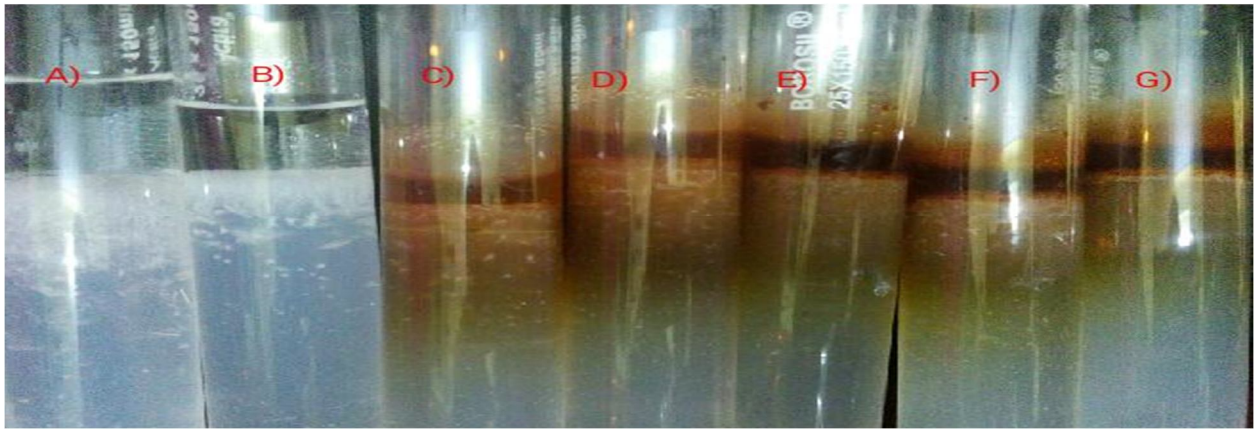

Fig. 1: The effect of Alpinia calcarata stem on struvite crystals in the gel method (A) without any additive (B) with the distilled water (C) with the 0.15% methanol extract (D) with the 0.25% methanol extract (E) with the 0.50% methanol extract (F) with the 0.75% methanol extract (G) with the 1.00% methanol extract after 7 days.

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Fig. 2: The harvested crystals of struvite obtained from Alpinia calcarata stem in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of methanol extract (d) with the 0.25% of methanol extract (e) with the 0.50% of methanol extract (f) with the 0.75% of methanol extract (g) with the 1.00% of methanol extract.

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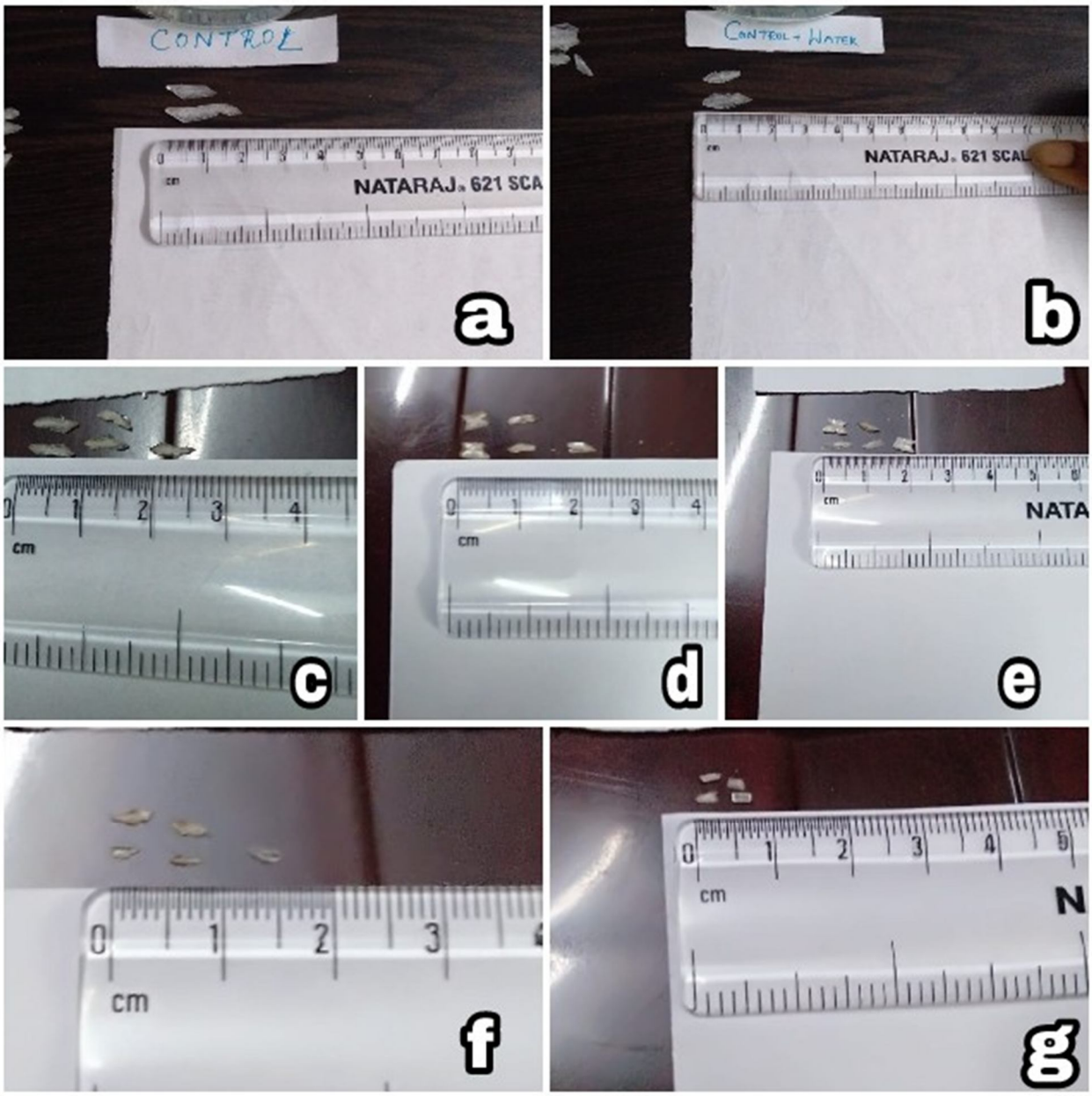

Fig. 3: The measurement of struvite obtained from Alpinia calcarata stem in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of methanol extract (d) with the 0.25% of methanol extract (e) with the 0.50% of methanol extract (f) with the 0.75% of methanol (g) with the 1.00% of methanol extract.

B. Characterization of Struvite crystals

The FTIR spectra of Struvite crystals obtained in the presence and absence of the Alpinia calcarata stem are shown in Fig. 4. In fig. 4 (a) without any additive, a strong band at 2365 cm^{-1} are due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1637 cm^{-1} is due to HOH deformation of water and the peak at 1442 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 756 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units. In the presence of 5% lemon juice Fig. 4(f), a band at and 2376 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1601 cm^{-1} is due to HOH deformation of water and the peak at 1440 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1005 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 756 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 567 cm^{-1} is due to the V_4 bending modes of the PO_4 units. In the presence of 5% orange juice

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Several researchers have reported crystallization characterization of struvite crystals using FTIR techniques [17, 31, 32]. The peaks shift from 2358 to 1674 cm^{-1} and from 1441 to 1438 cm^{-1} for HNH deformation modes of NH_4 units previously reported. The shifting further supports that the lemon juice can promote the formation of ammonium magnesium phosphate hexahydrate crystals and reduce the nucleation rate of struvite crystals as compared with orange juice.

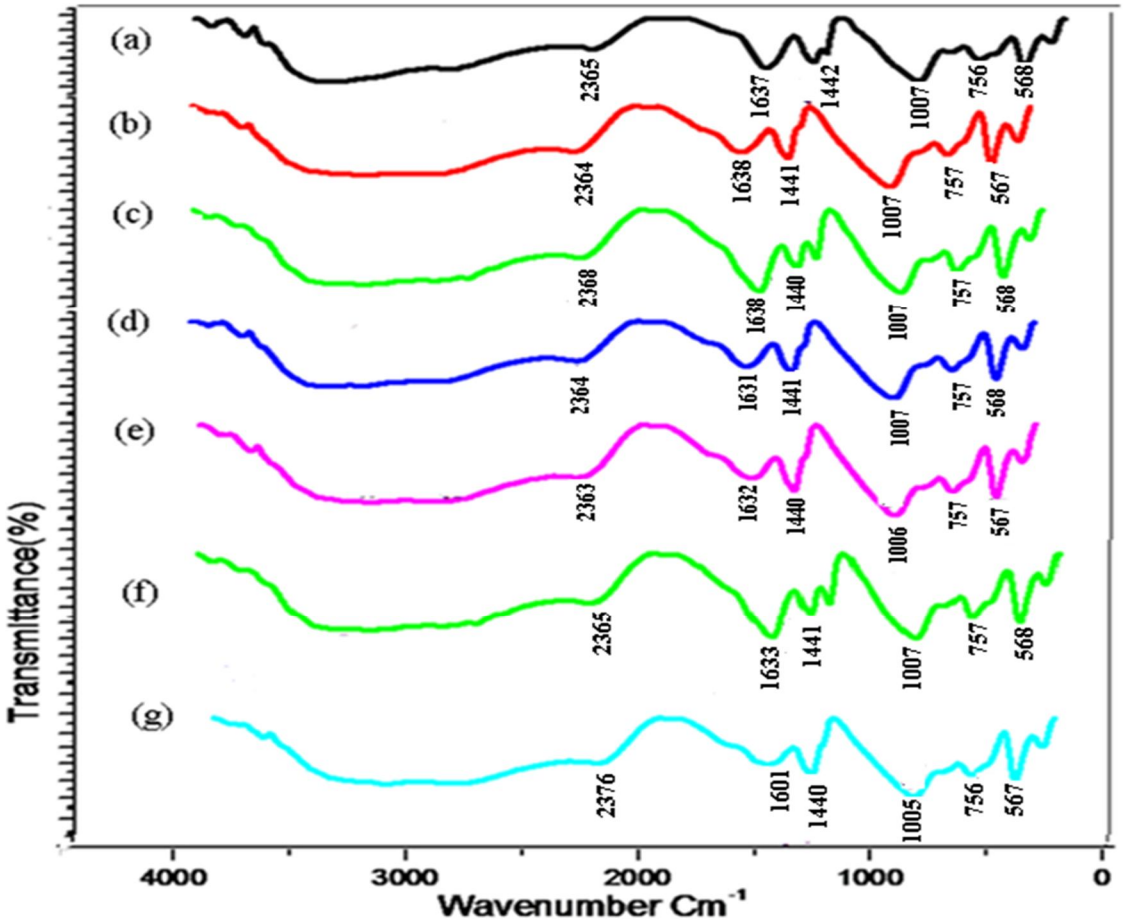

Fig. 4: The FTIR spectra of struvite obtained from Alpinia calcarata stem in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of methanol extract (d) with the 0.25% of methanol extract (e) with the 0.50% of methanol extract (f) with the 0.75% of methanol extract (g) with the 1.00% of methanol extract..

IV. CONCLUSION

Struvite crystals were grown by single diffusion gel growth techniques and characterized by FTIR techniques for the experimental confirmations of the grown crystal. With an increase in the concentration of methanol extract of Alpinia calcarata stem the weight of the formed crystals were gradually reduced from 2.83 g to 0.24 g in struvite crystals, respectively. FTIR techniques confirmed its functional groups of struvite crystals. One way ANOVA performed with treated and untreated crystal growth data obtained from struvite crystals showed significant differences ($p<0.05$). This study confirmed that the Alpinia calcarata stem extracts can promote the formation of ammonium magnesium phosphate hexahydrate crystals and treat urinary stone by inhibiting the formation of struvite crystals, a major component of triple phosphate urinary stone.

V. ACKNOWLEDGEMENT

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A. Conflicts of interests

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The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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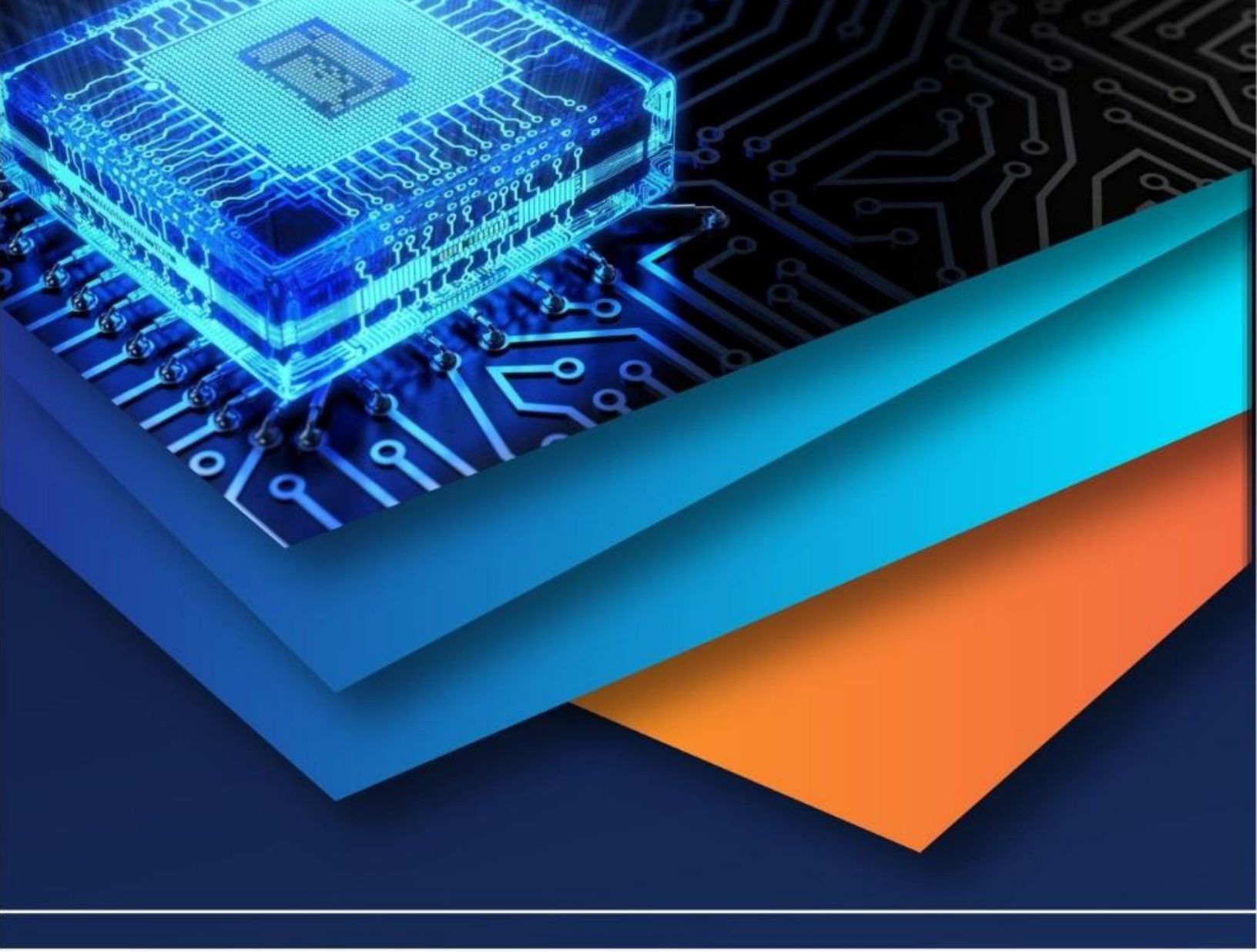

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