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Tridax Procumbens L.: Aqueous Solvent Isolation of a New Flavonoid, Phytoanalysis and Anti-Microbial Studies

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Abstract: Witnessing the efficacy with which wounds are healed, swelling is reduced and pain is relieved by using the fresh juice or an aqueous extract of Tridax procumbens L. , we were intrigued to undertake the Phytochemical screening, isolation of bioactive molecules possessing possible medicinal values and also anti-microbial activities of Tridax procumbens L., mailnly using water as the solvent. Different solvent systems have also been employed for the purpose of comparison under green conditions.

Keywords: Tridax procumbens L., traditional medicine, green extraction, phytochemical screening, anti-microbial.

I. INTRODUCTION

Plants play a vital role in the healthcare system, particularly in developing countries, because of their acceptability, economic viability and for aesthetic and cultural reasons. A number of modern drugs have been developed from plants, plant products, herbs and shrubs. In addition to high costs, synthetic drugs are commonly associated with adverse side effects and multi drug resistance to microorganisms [1]. Herbs and shrubs have been studied widely all over the world for their medicinal and other usability and limitations. Hence, renewed attention has been paid to biologically active isolated compounds from plant species used in herbal medicines [2]. They have been proved effective in the treatment of infectious diseases and mitigating many of the side effects often associated with synthetic drugs [3]. Positive response of plant based-drugs with less or no side effects might lie in the chemical structure of the natural products leading to the emergence of drug designing studies as a new field of research.

A. Review of the Plant

Tridax procumbens, a common weed, Fig. 1 to 3, known as coat buttons [4] or tridax daisy has several potential therapeutic

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activities like antiviral, anti-oxidant, antibiotic, wound healing activity, insecticidal and anti-inflammatory activity, etc. [5].

The leaf juice can be used to cure fresh wounds, to stop bleeding and also as a hair tonic. Its leaf extracts were known to treat infectious skin diseases in folk medicines. A mixture of Tridax procumbens and Allium sativum extracts used as a promising natural treatment for cutaneous leishmaniasis [6]. Ethanolic extract of the whole plant of Tridax procumbens showed significant anti-arthritic effect, antidiabetic and antihyperlipidemic effects [7]. The Tridax procumbens flower extract showed antidiabetic properties [8], petroleum ether extract of Tridax procumbens L. is effective in promoting hair growth [9]. The high moisture content of the leaves and stems of Tridax procumbens L. as a food complies to the index of water activity [10] and is used as a measure of stability and susceptibility to microbial contamination [11]. When dehydrated, T. procumbens can serve as a good source of protein [12]. This widely grown weed, available in all seasons and in most parts of the world [13] has been tested to possess a large number of chemical constituents which have been identified and isolated in flowers as well as other aerial parts of the plant and are well documented [14]. The flavonoids such as luteolins and quercetins are present in flowers [15]. The aerial parts (except flowering tops) possess various saturated and unsaturated fatty acids [16]. The plant also possesses phytosterols such as β-sitosterol, campesterol and stigmasterol which impart an anti-inflammatory property to it [17]. Six compounds isolated and structurally identified from Tridax procumbens Lin. [18] include three flavones: 8,3′-dihydroxy-3,7,4′-trimethoxy-6-O-β-D- glucopyranosyl flavone, 6,8,3′-trihydroxy- 3,7,4′- tri methoxy flavone and puerarin; one coumarin, esculetin and two triterpenoids: oleanolic acid and betulinic acid. A new bis-bithiophene named tridbisbithiophene, along with four known terpenoids: taraxasteryl acetate, beta-amyrenone, lupeol and oleanolic acid has been reported to be present in the plant [19]. It exhibits antiseptic, insecticidal and hair growth-promoting properties [20]. More commonly, the plant leaves are used to check haemorrhage from cuts, bruises and wounds [21]. The remarkable influence of healing wounds prompted its study of haemostatic activity [22]. A flavonoid glycoside, named 8,3'-dihydroxy-3,7,4'- trimethoxy-6-0-[u- L-rhamnopyranosyl-D-glucopyranoside flavone has been isolated which on hydrolysis gave the aglycone, 6,8,3'- trihydroxy- 3,7,4'- trimethoxyflavone. Oxylipin (3S)-16, 17-didehydrofalcarinol [23] has also been isolated from Tridax procumbens L.

B. Phytochemistry of Tridax Procumbens L.

The phytochemical screening of the leaves of T. Procumbents L. in various solvent systems revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. Higher carotenoid content in Tridax has been documented

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[24]. β-Carotene is used as a food colorant. T. procumbens has very high saponin content. Saponins are known to reduce the uptake of certain nutrients like glucose and cholesterol and may help lessening the metabolic burden on the liver [25]. Low tannin content in this plant was observed. Procumbetin, a flavonoid has been isolated from the aerial parts of Tridax procumbens L. [26] and characterised as 3,6-dimethoxy-5,7,2',3',4'-penta hydroxyl flavone-7-O-beta- D-glucopyranoside. Three new phytochemicals, a polyacetylene, 1,2-dihydrodendroarboreol B, an ionone derivative, (3S,5R,6S,7E)-3–tetradecanoate-5,6-epoxy- β-ionone and a flavonol diglycoside, quercetagetin-3,6,4′-trimethoxy-7-O- neo hesperidoside along with other thirty five known compounds with diverse structures were isolated from the ethanol extract of the aerial parts of Tridax procumbens L. [27]. Antibacterial activity of the crude extracts of Tridax procumbens L. has been reported to inhibit the growth and activity of various pathogens, both gram-positive and gram-negative bacteria [28]. Studies on antibacterial potential against E.Coli, Klebsiella pheumoniae, Proteus vulgaris, Bacillus subtilis and Staphylococcus aureus showed minimum inhibitory concentration ranges between 1.9 - 19.5 [29]. The plant exhibited broad spectrum antimicrobial activity, free and bound flavonoids of T. procumbens flowers were found to be more potent [30]. Hepatoprotective activity [31], Anti-diabetic activity [8] have also been reported with ethanolic extract of Tridax procumbens L. Marked beneficial effects of the extract of Tridax procumbens Linn. in anti-inflammatory and analgesic activities is presumably due to the presence of flavonoid and sterols which qualify it to be used as an effective anti-inflammatory and analgesic [32]. The ethanolic extract of the whole plant of T. procumbens exerts an anti-arthritic activity without exerting any side effects in arthritis [7], [33]. The ethanolic extract of the leaves of Tridax procumbens L. reduces the clotting time and possesses haemostatic activity and immunomodulatory effect [34], [35]. Aqueous extract of Tridax procumbens L. promote healing of both normal and immune-compromised (steroid treated) wounds [36]. Various extracts of the plant Tridax has shown anti-coagulant, anti-septic, insecticidal, parasiticidal properties and also to check haemorrhage from cuts, bruises, dysentery, diarrhoea and wounds [37], [38]. In the Indian system of medicine, Tridax procumbents L. is also commonly used in hypotensive conditions [39]. Tridax extracts (polyphenols) exhibit a very high degree of antioxidant activity which has been attributed to the possible hydrogen donors scavenging the free radical DPPH [40]. The aqueous and ethanolic extracts have anti-plasmodial activity against chloroquine resistant P. Falciparum parasites having considerably low toxicities to human RBCs [41]. The compound Lupeol, a phytochemical in dried leaves of Tridax procumbens L. having Rf value of 0.66 showed potential anticancer property [42]. Extracts of Tridax procumbens L. against the pathogenic fungal strains Aspergillusflavus and Aspergillusniger showed remarkable activity against A.

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niger making it a potential candidate for the formulations of antifungal drug for treatment of diseases caused by A. Niger [43]. Baicalein, a flavonoid present in Tridax procumbens L. leaves has been shown to be effective as an anti-tumor agent [44]. Anti-allergic properties of leaves of Tridax procumbens L. has been attributed to the presence of the flavonoid and Ellagic acid [45]. Nobiletin, a flavonoid present in the leaves of Tridax procumbens manifests anti-dementia, cognitive impairment improving properties [46] and antitumor properties [47]. The (+)-Catechin, a reported flavonoid constituent of Tridax procumbens L. leaves is antimicrobial [48], anti-allergic, anti-carcinogenic, anti-diabetic, antihypertensive, anti-inflammatory, anti- mutagenic and anti-obesity. This plant was also used for bronchial catarrh, dysentery, diarrhoea and as a remedy against conjunctivitis[49]. Tridax procumbens L. has been reported to promote hair growth and preventing falling of hair as a hair tonic [9.] Tridax procumbens L. has been explored for its potential use against general food spoilage and human pathogens so that new food preservatives may be developed [50]. Methanol extract of the whole plant of Tridax procumbens L. have been reported to exhibit significant leishmanicidal activity [6]. Tridax procumbens L. plant was also used as a good bioadsorbent for the removal of highly toxic ions of Cr (VI) from industrial wastewater and be recommended for bioremediation [36].

II. EXPERIMENTAL

A. Material and Methods

1) Collection of Plant Material: The whole plant of Tridax procumbens L. was collected locally from the campus of St. Xavier's High School, Meenakshi Nagar, Brahampur with 19o 20' N latitude and 84o 50' E longitude. The area is located by the side of "Sapua River" which carries the down-stream water from the Eastern Ghats. The sample was authenticated by Taxonomist in the Department of Botany, Khallikote University, Berhampur, Odisha and the voucher specimen was deposited in the Herbarium of Khallikote University (Ref. No. 47/16). Reagents Used

All the solvents used in this research work viz. ethanol, methanol, benzene, petroleum ether, chloroform and ethyl acetate were of highest purity and procured either from CDH or Merck or Ranbaxy and were used as such. Extraction and Isolation of Compounds

The plant under the present study is available wildly in the area and is liberally used by the local people as such, we under took the project to isolate the possible medicinal ingredients from the plant by using mainly the juice or the water extract of the plant. Various methods also have been adopted using different solvents for the purpose of comparison of the possible medicinal ingredients isolated from the plant Tridax procumbens (L.); the methods being depicted schematically, Schemes: 1 to 3.

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B. General Procedure

1) *Isolation Techniques:* The compounds were separately isolated using column chromatography and the purity of the individual compound was further tested on the TLC plates. In column chromatography, the stationary phase was packed in a cylindrical glass column with silica gel as adsorbent. Mixtures of petroleum ether and benzene in the ratios (9:1, 7:3 and 5:5) were mainly used. For Thin Layer Chromatographic studies mixture of benzene and ethyl acetate in the ratio 7:3 has been used. Benzene: chloroform: ethyl acetate (80:10:10) was found to be the best solvent system. Activated Silica gel G, (60-120 Mesh) was used for preparation of the plates. The dried chromatograms were developed in iodine chambers. The Rf values were calculated. Characterisation and structural elucidation of the compounds obtained were done using spectroscopic techniques. Antimicrobial activities of the isolated compounds were performed and are discussed.

2) *Spectroscopic Techniques:* Once a plant constituent has been isolated and purified, spectroscopic techniques were employed for structure elucidation of the isolated constituents [51]-[53]. UV- Visible Spectrophotometer- 1700(Shimadzu, Japan), at a range of 250 - 400 nm was used to detect the characteristic wavelength. Fourier Transform Infra-Red Spectroscopy (Shimadzu, Japan) was used operating in the range 4500 – 500 cm–1 using FT-IR grade KBr to prepare pellets. 1H-NMR spectroscopy and 13C-NMR Spectroscopy or CMR spectral studies were conducted using a Bruker AV- 400 at 298K (1H:400 MHz, 13C:100.6 MHz) while Mass spectral studies were followed using a Bruker Micro TOF-Q II Mass Spectrometer. An Ultrasonic Sonicator, PCI, India of 3.5 Lts was used.

C. Detection Method

1) *Preparation of Extracts:* Cleaned and shade dried sample of Tridax procumbens Linn plant was shredded into and the shredded pieces and preserved in air tight containers and 400 gm samples of which was soaked in 500 mL each of chloroform, ethyl acetate and methanol at room temperature for seven days with occasional shaking. The solvent from the total extract was filtered. The filtrate was used for phytochemical screening.

Phytochemical Screening and Antibacterial activity

The individual extract was subjected to phytochemical screening adopting standard procedures as described by Harborne [51]and Khandelwal [54] and presented in Table-1. Antimicrobial activities of the isolated compounds were performed and are discussed in the present work.

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D. Microorganisms

The bacterial strains Bacillus licheniformis M.T.C.C No.- 429, Escherichia coli M.T.C.C No.- 40, Proteus vulgaris M.T.C.C No.- 426, Pseudomonas aeruginosa M.T.C.C No.- 424, Shigella flexneri M.T.C.C No.- 1457, Bacillus subtillis M.T.C.C No.- 441, Staphylococcus aureus M.T.C.C No.- 87 and Staphylococcus epidermidis M.T.C.C No- 2639 were used for determination of antimicrobial activity. Routine sub culturing of the Gram positive bacteria was carried out on nutrient agar and of the Gram negative strains on bromothymol blue lactose agar [55]. The minimum inhibitory concentrations (MIC) of the isolated compound against the bacterial strains were determined by the agar dilution technique [56]. Desired amount of isolated compound was dissolved in sterile dimethyl sulfoxide (DMSO) to prepare the stock solution. Measured volumes of stock solution of the isolated compound were added aseptically to molten nutrient agar (Oxoid) in the concentrations (µg/mL): 0 (control), 10, 25, 50, 75, 100, 150, and 200 and poured into sterile petri dishes. The pH of the media was adjusted to 7.2- 7.4 and Spot Inoculation Method was carried out [57].

III. RESULTS AND DISCUSSION

A. Identification of the Extracted Compound

The compounds isolated from Tridax procumbens L. gave the same Thin Layer Chromatography Rf value of 0.81 against iodine vapour and the spectral data indicated that the same product was obtained by various methods as described in Schemes 1 to 3.

B. Spectral Analysis of Compound (1)

1H- NMR (CDCl3); ð PPM: 0.9(m, -CH3), 1.3(s, -CH2), 1.75(-CH2), 2.3(dd, H2), 3.9(s, -OCH3), 4.5(Heptate, OH), 7.25 (m, H2',3'), 7.35(s, H5'), 7.5(dd, H6), 7.7(dd, H8). 13C- NMR (CDCl3); ð PPM: 10.95(-CH2), 14.03(-CH2), 14.11(-CH2), 23.0(-CH2), 29.74(CH), 30.4(-CH2OH), 38.77(-OCH3), 68.12(glu), 76.73(glu), 77.05(glu), 77.37(glu), 128.8(Ar-C), 130.84(Ar-C), 132.49(Ar-C), 167.73(>C=O). Mass (m/e): 414, 540. UV: 275, 290 nm. IR (ν, Cm-1): 3445.01 (-OH), 2957, 2862.49 (C-H streching), 1730.22 (>C=O), Aromatic (1590.36, 1459.21, 1380.13).

Basing on the above spectral studies of the isolated compound from Tridax procumbens L., the molecular formula corresponds to C24H28O10. The 1H NMR signals, the 13C NMR spectrum revealing 24 carbon signals and the NMR spectral data suggest that it is a flavonoid. The mass spectra, 1H and 13C NMR data indicate the compound to be 7-Ethyl-5-hydroxy-4'-methoxy flavone-3-glucopyranoside, which has been reported for the first time from this species. IUPAC name: 7-Ethyl-2,3-dihydro-5-hydroxy-2-(4-methoxyphenyl)-3-(tetrahydro-4,5,6-trihydroxy-2-(hydroxymethyl)-2H-pyran-3-yloxy) chroman-4-one, Fig. 4.

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C. Phytochemical Analysis

Phytochemical assay of Tridax procumbens L. extracts in various solvents have been performed following standard methods and the results are presented in Table- 1. The amounts of the various phyto-constituents that were observed are denoted as +++, ++, + or – depending on their relative concentrations. However, the quantitative amounts are not detected.

D. Antimicrobial Assay

Antimicrobial Activity Study of Isolated Compound (Determination of MIC Value) by Agar Dilution Method: All the eight bacterial strains were made to grow in nutrient broth and after incubation at 37 0C for 18 hours following spot inoculation on nutrient agar plates, which contained increasing amounts of the drug. The nutrient agar plates that lacked the drug but contained equal volumes of the solvent were taken as the control. The inhibitory pattern of the in vitro study is presented in Table 2. Table 2 shows that out of 08 bacterial strains, Escherichia coli MTCC NO-40 and Pseudomonas aeruginosa MTCC No-424 were inhibited at the concentration of 50µg/mL. The bacterial strain Proteus vulgaris MTCC No-426 was inhibited at the concentration of 75µg/mL. The bacterial strain Shigella flexneri MTCC No-1457 was inhibited at the concentration of 100µg/mL. The remaining bacterial strains were not inhibited by the isolated compound up to the concentration of 200µg/mL. The results of antibacterial activity indicate that the isolated compound shows significant activity against “Gram –ve” bacteria and the MIC values were within the range of 50 to 100µg/mL.

IV. CONCLUSION

Our present study showed that the whole plant extract of Tridax procumbens L. rich in antioxidants, phenolics, terpenes, sitosterol, alkaloids, steroids, flavonoids etc. contribute respectively to the antioxidant, antimicrobial, analgesic, anti-inflammatory, antidiabetic, antifungal, antileishmanial, wound healing, immunomodulatory, hair tonic, antidiuretic, antiviral, anti-allergic activities as well as treatment of a variety of diseases due to its antimicrobial activities and anticancer activities which has also been reported earlier. The presence of terpenoids in this plant can widely be used in herbal medicine. Isolation of 7-Ethyl-2,3-dihydro-5-hydroxy-2-(4-methoxyphenyl)-3-(tetrahydro-4,5,6-trihydroxyl-2-(hydroxyl methyl)-2H-pyran-3-yloxy)chroman-4-one for the first time in aqueous solvent from Tridax procumbens L. has been achieved. In water Tridax procumbens L. has shown an unprecedented susceptibility to Gram -ve microorganism. The +ve inhibition effect with the Gram -ve microorganism Pseudomonas aeruginosa is notable. It proves to be a promising candidate for a potential drug in combating a series

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of common ailments which may add immensely to socio-economic benefits in general and the health condition of the poor and needy in particular. Further probe with animal studies will help getting an insight into the details of drug doses, drug delivery system and mode of action.

All the solvents used, the techniques adopted, cost involved and the disposal of the plant waste after extraction, laboratory and environmental safety considerations justifies the method to be treated as a greener method in comparison to the other methods adopted for the similar study of the plant.

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Table 1: Phytochemical Assay of Tridax Procumbens L. Extracts in Various Solvents

Chemical Name	Aqueous extract	Aqueous extract (Hot)	Methanolic extract	Ethyl acetate extract	Chloroform extract
Flavonoids a. b. c. d.	+ +	+ + + +	- - - -	- - - -	+ ++ - -
Tannins a. b.	+ +	++ ++	- -	- -	+ ++
Steroids	++	++	-	-	-
Cardial glycosides	++	++	+++	+	++
Saponins		+	-	-	-
Phenols	+	+++	-	-	-
Coumarins	++	++	+	+	+
Alkaloids a. b.		+++ +++	+++ -	+++ +	+++ -
Amino acids		+++	+	-	-
Anthocyanins	-	-	-	-	-
Diterpenes		-	++	+++	+
Carbohydrates		+	+	-	++
Lucoanthocyanins		-	-	-	-
Proteins	+	+	+	-	+
Emodins	-	-	-	-	-
Phytosterol	+	+	-	-	-
Phlobatannins	+	+	-	-	-

Note : '+' indicates presence '-' indicates absence

Table 2: Minimum Inhibitory Concentration (MIC) of Isolated Compound against Different Bacterial Strains.

Sl. No.	Name of the Bacteria	Growth on nutrient agar containing different concentrations of isolated compound (µg/ml)							
		0	10	25	50	75	100	150	200
1	Bacillus licheniformis M.T.C.C No - 429	+	+	+	+	+	+	+	+
2	Bacillus subtillis M.T.C.C No - 441	+	+	+	+	+	+	+	+
3	Escherichia coli M.T.C.C No - 40	+	+	+	–	–	–	–	–
4	Proteus vulgaris M.T.C.C No - 426	+	+	+	+	–	–	–	–
5	Pseudomonas aeruginosa M.T.C.C No - 424	+	+	+	–	–	–	–	–
6	Staphylococcus aureus M.T.C.C No - 87	+	+	+	+	+	+	+	+
7	Staphylococcus epidermidis M.T.C.C No -2639	+	+	+	+	+	+	+	+
8	Shigella flexneri M.T.C.C No - 1457	+	+	+	+	+	–	–	–

'0' stands for plain nutrient agar without the drug serving as control '+' stands for growth and '-' stands for no growth.

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Figure Captions

Fig. 1 Tridax procumbens L Flower

Fig. 2 Tridax procumbens L Leaves

Fig. 3 Tridax procumbens L Plant

Fig. 4: 7-Ethyl-5-hydroxy-4'-methoxy flavone-3-glucopyranoside

Fig. 1.

Fig. 2.

Fig. 3.

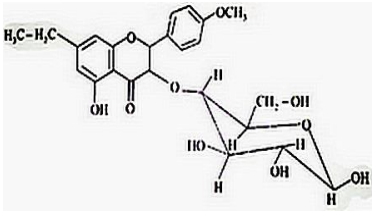
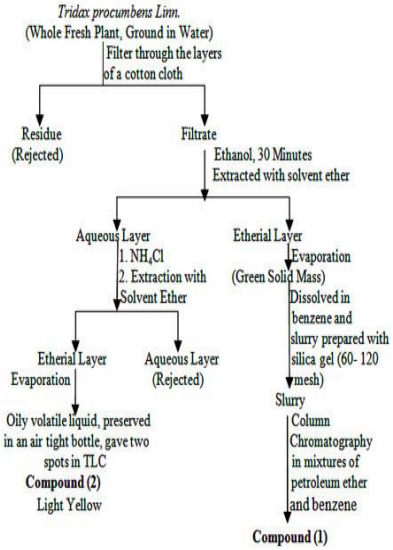
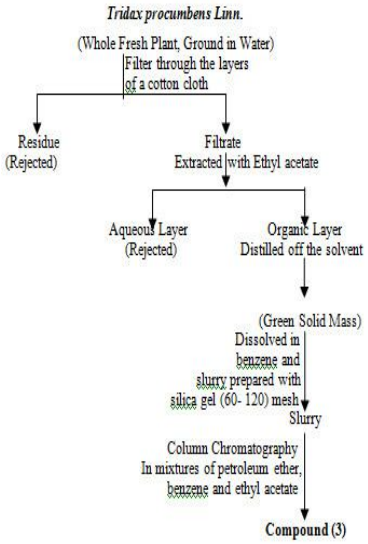


Fig. 4.

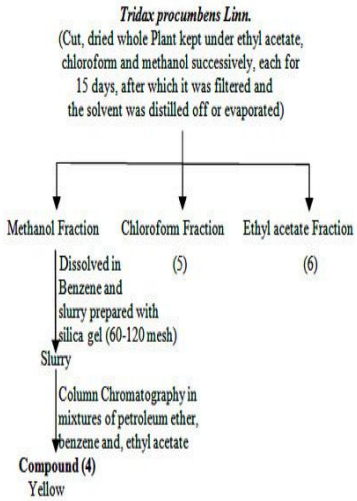
Scheme: 1



Scheme: 2



Scheme: 3



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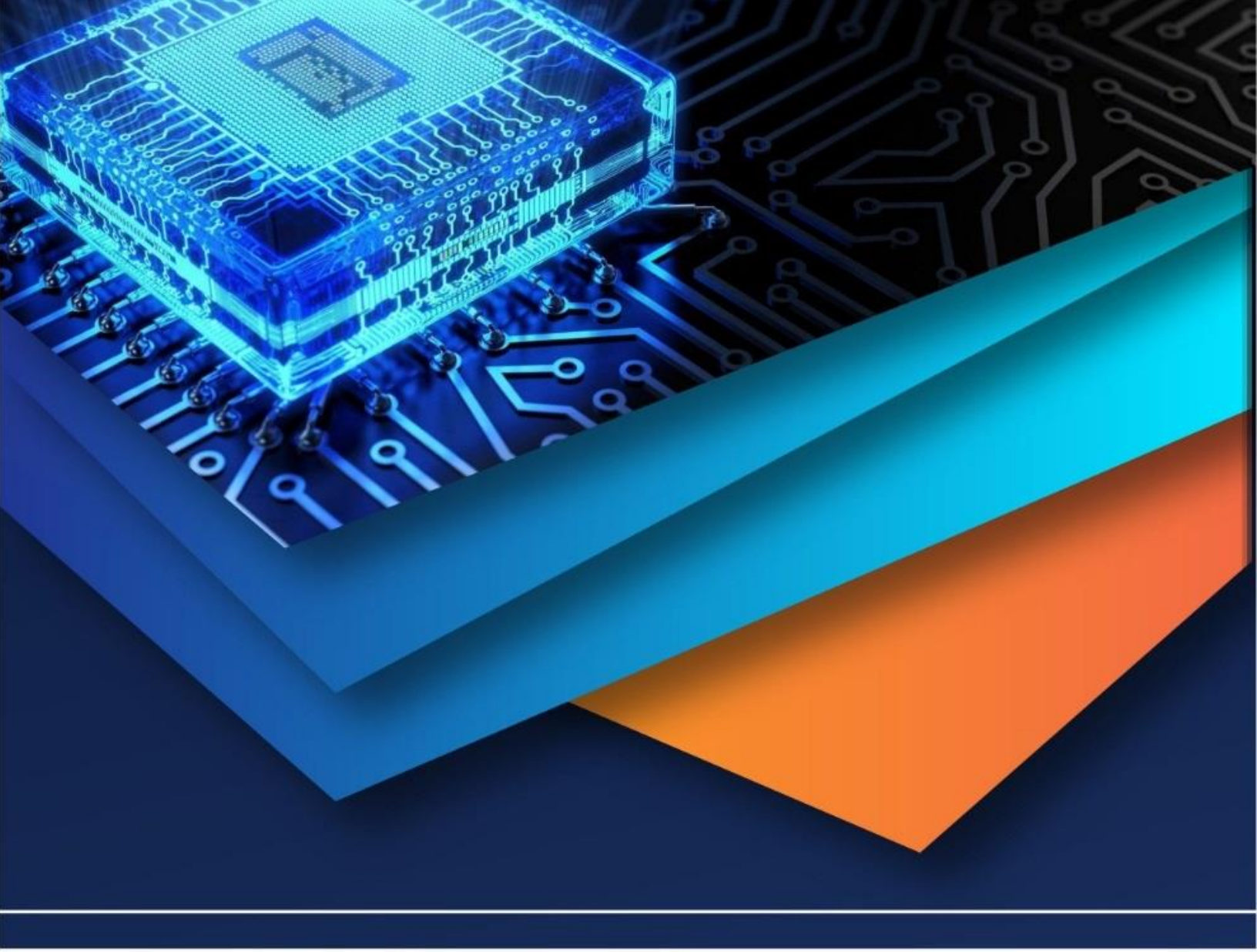

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