iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** VI **Month of publication:** June 2017

DOI:

www.ijraset.com

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Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Analysis of Total Phenol, Cellulose and Tannin Content by Using Different Parameters in Ethanol Extract of Pomegranate Peel

Vijayalakshmi. T[1], Dhanarajan. M. S[2], Chandra Mohan. A[3]
Research Scholar[1], Guide[2], Professor[3], PG Research, Department of Biotechnology and Biochemistry,
Jaya College of Arts and Science, Affiliated to University of Madras.

Abstract-: Pomegranate (Punicagranatum L.) is considered one of the oldest known edible fruits. Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30%of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids and tannins. Pomegranate fruit and its peel exhibit a high antioxidant potential. Pomegranate peel attracts attention due to its apparent wound healing properties, Antioxidative activity has often been associated with a decreased risk of various diseases. The purpose of the present study was to evaluate the effect of using different parameters with ethanol extract and estimate the efficiency of effective compounds, such as polyphenolic, cellulose and tannin compounds from the pomegranate peel extracts.
Keywords--Pomegranate peel extracts, polyphenolic, cellulose and tannin

I. INTRODUCTION

Pomegranate (Punica granatum L. Punicaceae; the common name is derived from Latin words ponusandgranatus), a seeded or granular apple, is a delicious fruit consumed worldwide. The fruit is native to Afghanistan, Iran, China and the Indian sub-continent. The ancient sources of pomegranate linked Iran to Pakistan, China and eastern India, where pomegranate shad been under cultivation for thousands of years.

Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30%of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compound sarecon-centrated in pomegranate peel (PoP) and juice, which account for 92% of the antioxidant activity associated with the fruit [1], [11] and [17].

The mechanism of antimicrobial activity of Pomegranate peel phenolics involves precipitation of membrane proteins resulting in microbial cell lysis. The ethno pharmacological profile of PoPx makes it a valuable traditional asset due to its antimicrobial and anti-mutagenic properties. Moreover, the phyto- chemical concentration of PoP is high enough to be effective without further enrichment with the extracts of any other fraction of the fruit [13].

A. Traditional medicinal uses

A variety of cultures and traditions in both the developing and developed worlds recommend pomegranate peel to treat common health problems. Traditionally, aqueous PoP extract is obtained by boiling for 10–40min. The extract has been used to treat diarrhoea, dysentery, and dental plaque, in addition to being used as adouche and enemaagent [8]. Similarly, diarrhoea, intestinal worms, bleeding noses and ulcers have been treated in Indian Subcontinent with dried PoP.

Pomegranate peel attracts attention due to its apparent wound healing properties [3], immune modulatory activity [5] and antibacterial activity [10] antiatherosclerotic and antioxidative capacities [15]. Antioxidative activity has often been associated with a decreased risk of various diseases [16]. The peel packs some of the weight boosting and health enhancing effects of antibiotics and hormones without the detrimental effects and it may yield meat with higher level of beneficial antioxidants [14]. Pomegranate Ellagitannin have been identified as the active antioxidant compound and anticancer activities responsible for protecting low density lipoprotein, cholesterol from

In the last few years the identification and development of phenolic compounds or extracts from different plants has become a major area of health and medical related research [4]. The present study is undertaken to know the nutritional importance and suitability of

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de-tanninated pomegranate peel, a by-product of pomegranate juice industry, as a cattle feed supplement.

II. MATERIALS AND METHODS

A. Collection of pomegranate peel

Collection, separation and drying of pomegranate peel. The orange fruits were purchased. The peels were manually separated from the fruit. The peels were shade dried. The dried peels were collected and ground well to form a powder. The powdered orange peel was stored in an airtight container and used for various tests.

B. Preparation of the peel extract

Preparation of the extracts was assessed by the following methods. One gram of dried bitter orange peel powder was extracted with 20 ml of aqueous, ethanol was soaked overnight at room temperature. The sample was then filtered through Whatman.No.1 paper in a Buchner funnel.

The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extract was approximately 100%. The extracts were used for further tests.

Qualitative phytochemical analysis: The phytochemical tests were carried out using standard methods of analysis of tannins, saponins, quinones, flavanoids, glycosides, cardiac-glycosides, terpenoids, phenols, coumarins, steroids, alkaloids, anthocyanin and betacyanin

C. Materials required

1) Acetic/Nitric Reagent: Mix 150ml of 80% acetic acid and 15mL of concentrated nitric acid.
2) Anthrone: Dissolve 200mg anthrone in 100mL of ice-cold 95% sulphuric acid. Prepare fresh and chill for 2h before use.
3) 67% sulphuric acid
4) Folin-Denis Method: This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group.
5) Sodium carbonate
6) Tannic acid
7) Folin-ciocalteau reagent
8) Distilled water
9) *Methods*

D. Sonication

Sonication is the process of converting an electrical signal into a physical vibration that can be directed toward a substance. Sonicators are vital lab equipment and are used for a number of purposes. Sonication is usually performed to break apart compounds or cells for further examination. The vibration has a very powerful effect on solutions, causing their molecules to break apart and cells to rupture. A prime example is in DNA testing, where the cells that may contain DNA information are subjected to sonication to break them apart and release the DNA proteins so they can be tested.

The primary part of a sonication device is the ultrasonic electric generator. This device creates a signal (usually around 20 KHz) that powers a transducer. This transducer converts the electric signal by using piezoelectric crystals, or crystals that respond directly to the electricity by creating a mechanical vibration. This vibration, molecular in origin, is carefully preserved and amplified by the sonicator, until it is passed through to the probe

The sonication probe transmits the vibration to the solution being sonicated. This probe is a carefully constructed tip that moves in time with the vibration, transmitting it into the solution. The probe moves up and down at a very high rate of speed, although the amplitude can be controlled by the operator and is chosen based on the qualities of the solution being sonicated

E. Magnetic Stirrer

A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic field to cause a stir bar (also called "flea") immersed in a liquid to spin very quickly, thus stirring it. The rotating field may be created either by a rotating magnet or a set of stationary electromagnets, placed beneath the vessel with the liquid.

Magnetic stirrers are often used in chemistry and biology, where they can be used inside hermetically closed vessels or systems, without the need for complicated rotary seals. They are preferred over gear-driven motorized stirrers because they are quieter, more efficient, and have no moving external parts to break or wear out (other than the simple bar magnet itself). Magnetic stir bars work well in glass vessels commonly used for chemical reactions, as glass does not appreciably affect a magnetic field.

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The limited size of the bar means that magnetic stirrers can only be used for relatively small experiments, of 4 liters or less. Stir bars also have difficulty in dealing with viscous liquids or thick suspensions. For larger volumes or more viscous liquids, some sort of mechanical stirring is typically needed. Because of its small size, a stirring bar is more easily cleaned and sterilized than other stirring devices. They do not require lubricants which could contaminate the reaction vessel and the product. Magnetic stirrers may also include a hot plate or some other means for heating the liquid.

F. Quantitative phytochemical analysis

1) *Estimation of Total phenolic content:* Total Phenolic content (TPC) in the ethanol extracts was determined using the Folin-Ciocalteu reagent method [9]. This method depends on the reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm using spectrophotometer. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 5ml of Folin-Ciocalteu Reagent were added. The mixture solution was vortexed and incubated in the dark for 3 minutes, respectively. To the incubated content 5 ml of sodium carbonate (75g/L) solution was added to the above content and mixed thoroughly. The reaction content was incubated in the dark for 1 hour. The absorbance was read at 765 nm. Blank was maintained with 5 ml Folin-Ciocalteu reagent, 1 ml ethanol and 4 ml sodium carbonate solution. The concentration of total phenolic content in the extract was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract. Gallic acid stock solution was prepared to the concentration of 1 mg/ml. Serial dilution was carried out; gallic acid solution was dissolved in ethanol. A linear dose- response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm.
2) *Estimation of Total cellulose content:*Add 3mL acetic/nitric reagent to a known amount (0.5g or 1g) of the sample in a test tube and mix in a vortex mixture. Place the tube in a water bath at 100°C for 30min. Cool and then centrifuge the contents for 15-20min. Discard the supernatant and Wash the residue with distilled water. Add 10mL of 67% sulphuric acid and allow it to stand for 1h. Dilute 1mL of the above solution to 100mL. To 1mL of this diluted solution, add 10mL of anthrone reagent and mix well. Heat the tubes in boiling water bath for 10min. Cool and measure the color at 630nm. Set a blank with anthrone reagent and distilled water. Take 100mg cellulose in a test tube and proceed from step No. 6 for standard. Instead of just taking 1mL of the diluted solution (Step 7) take a series of volumes (say 0.4 to 2mL corresponding to 40-200mg of cellulose) and develop the color.
3) *Estimation of total tannins content:* Total Tannin content in the ethanol extract was determined by Folin–Denis method [12] with minor modifications. Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. To 0.1ml of each extract, 1ml of distilled water was added and then mixed with 0.5 ml of Folin–Denis reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (1mg/ml). A calibration curve was generated using various concentrations of Tannic acid (20 - 120µg) was obtained. Blank consist of all the reagents, except for the extract or standard solution is substituted with 0.1 ml of water. Results were expressed as mg of Tannic acid equivalent/g of dry weight (mg TE/g) of extracts.

III. RESULT AND DISCUSSION

The preliminary Phytochemical screening and analysis carryout with standard procedures of ethanol solvent in orange peel extracted and with different parameter a) ultrasonicator b) magnetic stirrer at various temperatures (37ºc and 50ºc) evaluated the presence of phytochemicals such as Phenol, cellulose and tannins, [6].

The peel samples shows the strong presence of Carbohydrates, Tannins, and Phenols in ethanol extracts. Saponin, coumarins and steroids are present in the methanol extract. Based on the presence of phytocompounds the further estimation will carried out phenol, cellulose and tannin.

As phytochemicals often play an important role in plant defence against prey, microorganism, stress as well as interspecies protections, these plant components have been used as drugs for millennia and hence, screening of phytochemicals serves as the initial step in predicting the types of potential active compounds from plants [2].

These compounds present in a variety of medicinal plants and fruits have significant application against human. Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases [7].

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Volume 5 Issue VI, June 2017
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A. Estimation of Total phenolic content (TPC)

The concentrations of total phenolic content in the extracts were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract the total phenolic content of the pomegranate of peel extract samples were determined using the Folin-Ciocalteu reagent method. The reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm was measured spectro-photometrically.

The results revealed the presence of highest total phenol content in the ethanol extract of pomegranate peel in different parameter at various temperature in 37˚C and 50˚C is shown in Fig. 1. The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD value for ultra sonicator at 37˚C in 10mg is (1.1791), where as in magnetic stirrer the highest OD value is seen at 50c in 10 mg is (1.4647). The highest total phenolic content were obtained in Ultra sonicator at 37 in 10mg. Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenols are able to scavenge reactive oxygen species due to their electron donating properties.

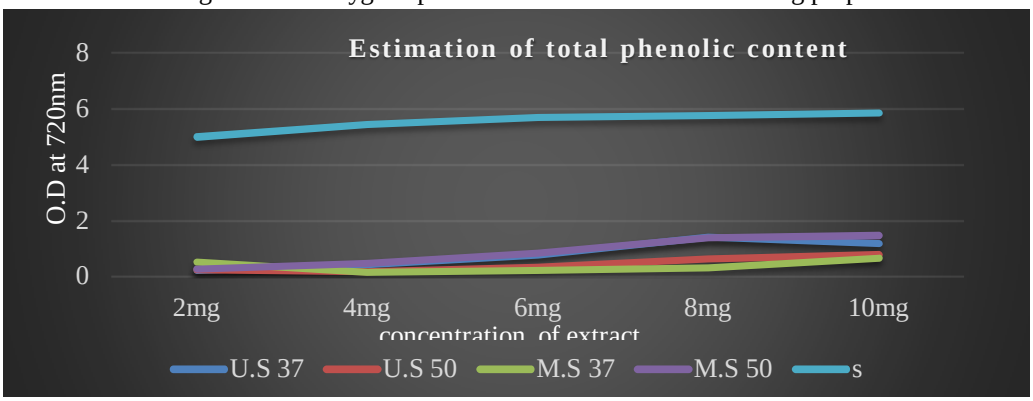

Fig. 1 Estimation of total phenolic content from ethanol extracts

B. Estimation of Total cellulose content (TCC)

The total cellulose content of the pomegranate peel extract samples were determined using the anthrone method. The reduction of AR by cellulose to a mixture of blue oxides which have maximal absorption at 630nm was measured spectrophotometrically. The result revealed the presence of highest total cellulose content in the ethanol extract of pomegranate peel in different parameters at various temperature in 37˚c and 50˚c is shown in the Fig. 2.

The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg and 10mg showed increasing increasing OD value respectively. The highest OD value of ultrasonicator at 37˚c in 10mg is (1.5519), where as in magnetic stirrer the highest OD value seen at 50˚c in 8mg (1.1295). The highest total cellulose content were obtained in ultra sonicator at 37˚c in 10mg.

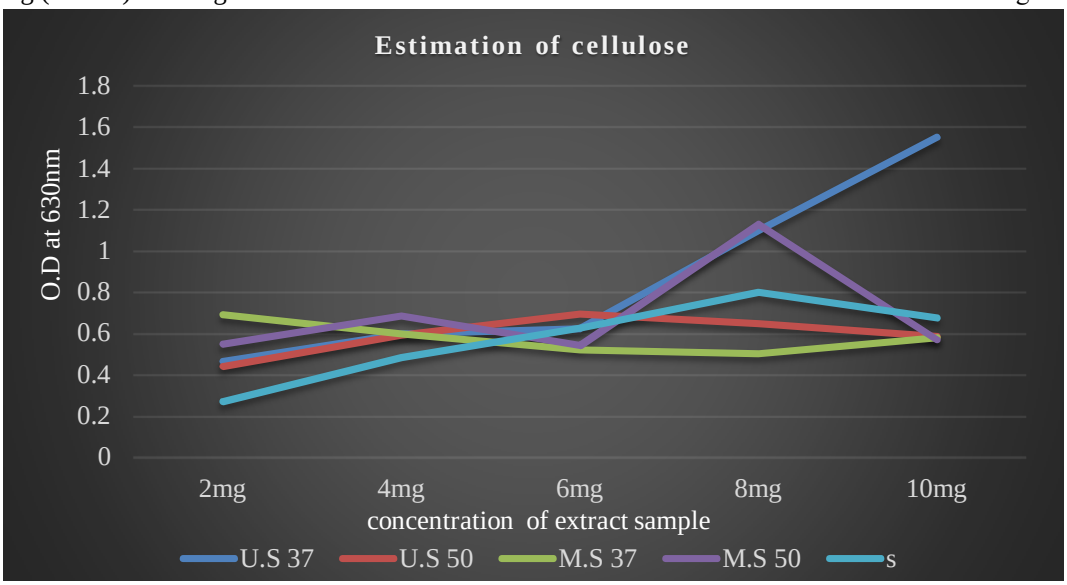

Fig. 2 Estimation of total cellulose content from ethanol extracts

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Volume 5 Issue VI, June 2017
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

C. Estimation of total Tannin (TTC)

The concentration of total tannin content was expressed as mg of Tannic acid equivalent/g of dry weight (mg E/g) of extracts. The total Tannin content of the pomegranate peel extract samples were determined using by Folin–Denis method. The reduction of FDR by tannin to a mixture of blue oxides which have a maximal absorption in the region at 700nm was measured spectrophotometrically.

The result revealed the presence of highest total-Tannin content in the ethanol extract of pomegranate peel in different parameters at various temperature in 37˚c and 50˚c in shown in Fig. 3. The increasing concentration of ethanolic extract, 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD value of ultrasonicator at 37˚c in 10mg is (0.6507), where as in magnetic stirrer the highest OD value is seen at 50˚c in 10mg (0.7009). The highest total tannin content were obtained in magnetic stirrer at 50˚c in 10mg.

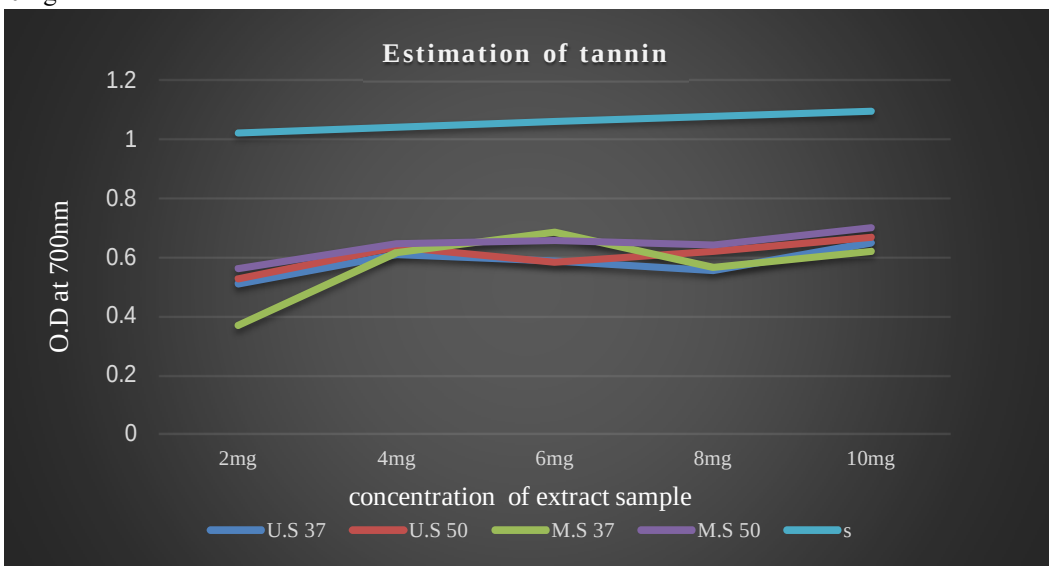

Fig. 3 Estimation of total tannin content from ethanol extracts

IV. CONCLUSION

Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. Pomegranate fruit and its peel exhibit a high antioxidant potential.

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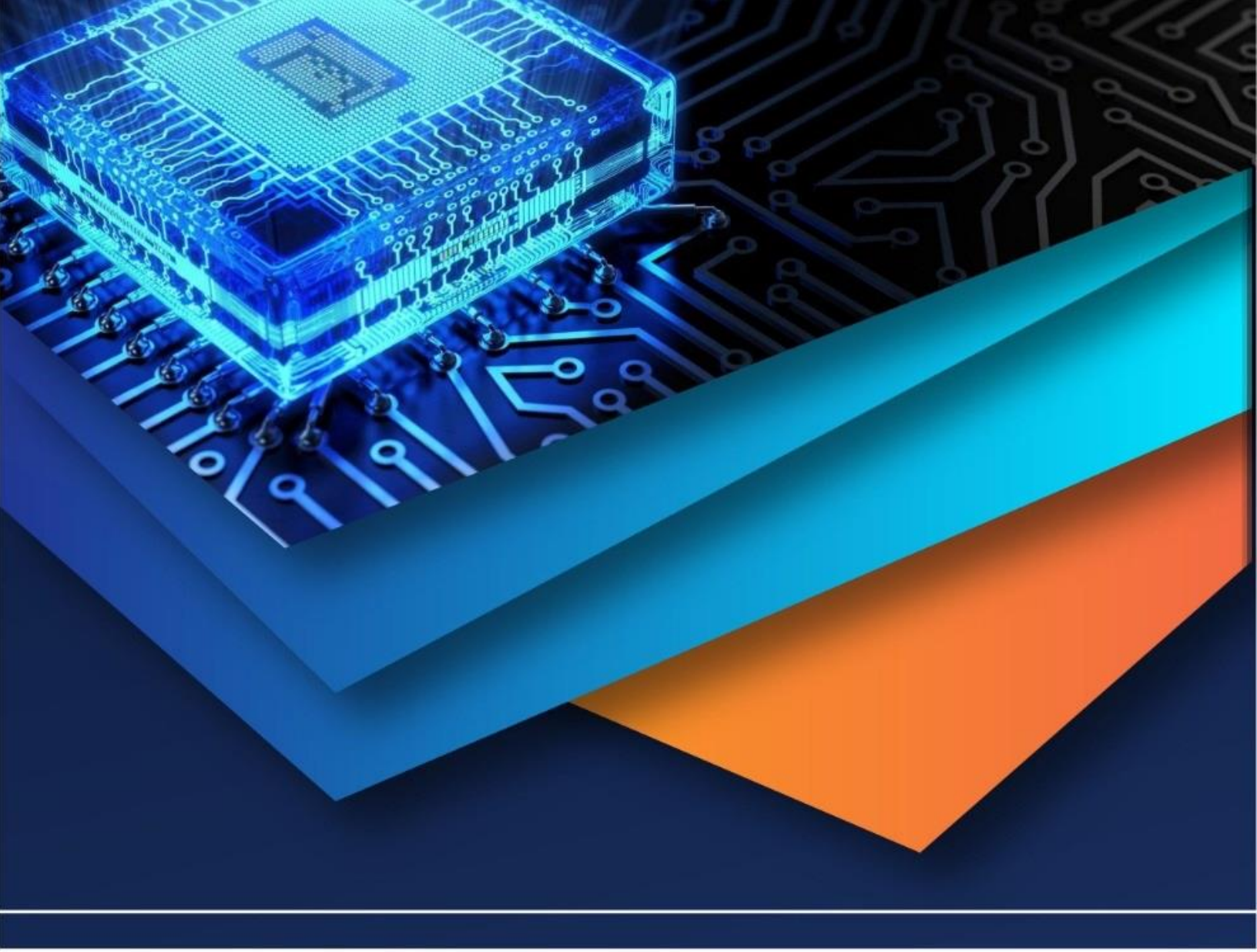

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