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A Study on Estimation of Phytochemical, Nutrition and the Antioxidant Activity of Dried *Madhuca longifolia* Flowers

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Abstract: ***Food derived phytochemicals possess various therapeutic properties to benefit the health of individuals against the illness or diseases caused due to oxidation of free radicals. Mahua (Madhuca longifolia) flowers are one of the most efficient therapeutic agents used through the ancient age mainly among the tribal communities. The present study aimed to analyze the phytochemicals content (both qualitative and quantitative) and antioxidant activity against DPPH (2, 2-diphenyl-1-picrylhydrazyl) of dried mahua flowers using aqueous extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, terpenoids, cardiac glycosides, coumarins, and steroids whereas anthraquinone was absent in the qualitative analysis. The total phenol and total alkaloids content was found to be 107 and 12.28 mg/g dry extract respectively depicting it as an abundant source. The aqueous extract at the concentration of 8.64 µg/ml showed maximum inhibitory activity (IC_{50}) against DPPH (2, 2-diphenyl-1-picrylhydrazyl). They are also rich in key nutrients such as carbohydrate (48.79 g/100g), fibre (19.16 g/100g) and iron (8.064 mg/100g) of the dry fruit. The reported data reveals mahua flowers to be an excellent source of phytochemical content, nutrition content and antioxidant property emphasizing its therapeutic potential. Thus, further investigation in this regard may contribute to its utilization in the field of nutraceuticals and pharmaco-therapy aiding against various chronic health conditions.***

Keywords: Edible flowers, Mahua, Nutraceutical, Phytochemical.

I. INTRODUCTION

Phytochemicals are naturally occurring, biologically active chemical compounds present in plants, which provide health benefits for humans further than those attributed to macro- and micro nutrients. Secondary metabolites derived from plants such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, carotenoids, etc., possess commercial application as drug, flavor, fragrance, etc. These fine chemicals are utilized in their purified form, extracted from different plant materials. Recent research studies conducted on these phytochemicals demonstrates that it can protect humans against diseases such as cancer, cardiovascular, arthritis, diabetes, aging etc., (https://www.atsdr.cdc.gov/mmg/mmg.asp/). *Madhuca longifolia* commonly referred to as mahua or butternut tree (Akshatha, et.al., 2013). It is a deciduous tree, ranging from 10-15 m tall with a spreading, dense, round and shady canopy. The species is distributed in the northern, central and southern parts of peninsular India, Sri Lanka and Burma. Mahua tree can grow on a wide variety of soils but thrives best on sandy soil (Annalakshmi, et.al., 2012). Mahua flowers are found in clusters, usually present at the tip of the branches, white in color, 2 cm length, pointed, sweat scented and fleshy (Arun, et.al., 2017).

Mahua flowers are also a potent source of nutrients such as total sugar, carbohydrates, protein, fat, etc. paper chromatography of un-hydrolyzed mahua flowers extract shows the presence of maltose, sucrose, glucose, arabinose, fructose, and rhamnose while galacturonic acid was found in the hydrolyzed extract. They also contain the amino acids such as lysine, aspartic acid, glutamic acid, threonine, valine, tryptophan, phenylalanine, isoleucine, leucine, and proline (Patel and naik, 2010). They are traditionally beneficial in heart diseases, burning sensation, and ear complaints. The flowers, benefits patients with piles on consumption in fried form in ghee (Behera, et.al., 2016). Mahua flowers can be used as tonic, analgesic, and diuretic. It has also been traditionally used as a cooling agent, tonic, aphrodisiac galactogogue, astringent, demulcent and anti-helminths, acute and chronic tonsillitis, pharyngitis as well as bronchitis (Boly, et.al., 2016). Its' juice is rubbed for oleation in skin diseases.

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The flower decoction effectively quenches thirst and is also a valuable remedy for pitta derived diseases. The powder works well with ghee and honey as a general tonic. In raktapitta, the fresh juice of flowers is used to arrest the bleeding (Akshatha, et.al., 2013). Mahua flowers exhibits various therapeutic properties, such as antioxidant activity, analgesic activity, antipyretic activity, antiulcer activity, anti-inflammatory activity, anthelmintic activity, antimicrobial activity, hepatoprotective activity and anticancer activity as depicted in the earlier studies. With increase in concentration of flower extract and ascorbic acid, the ferric reducing antioxidant power increases resulting in the antioxidant activity (Indu and Annika, 2014). The aqueous and alcoholic extracts derived from *Madhuca longifolia* flowers reported to possess analgesic effect (Irondi, et.al., 2013). Administration of 300 mg/kg body weight of ethanolic extract of *M. longifolia* flowers, showed a significant ($p<0.01$) antiulcer activity which could be attributed to its' rich phytochemicals content (Kalaivani and Jagadeesan, 2013). The methanolic extract of mahua flowers demonstrated the maximum anthelmintic activity against *Pheretimaposthuma* (Indian Earthworm) when compared to ethanolic extract (Katiyar, et.al., 2011). The alcoholic extract of flowers revealed significant antimicrobial activity against microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Aspergillus oryzae* and *Aspergillus niger* (Kalaivani and Jagadeesan, 2013). Administration of 200 mg/kg of methanolic extract of flowers showed hepatoprotective activity against paracetamol-induced hepatotoxicity in rats (Umadevi, et.al., 2011).

Drying a small quantity of medicinal plants without auxiliary energy is one of the commonly followed methods at domestic level. Drying is a method for post-harvest preservation of medicinal plants, and to be accomplished as soon as possible after harvesting in-order to increase the quality of plants and to prevent the losses due to contamination (Irondi, et.al., 2013). Considering the multiple uses of mahua flowers the present study was undertaken to study the nutrition composition, qualitative and quantitative (selected phytochemicals) analysis of phytochemicals of dried mahua flowers and its antioxidant activity against DPPH (2, 2-diphenyl-1-picrylhydrazyl). The quantification of phytochemicals in the present study is the novelty of the work carried out.

II. MATERIALS AND METHODS

A. Collection And Processing Of Dried Mahua Flowers

Fresh Mahua flowers were collected in the month of April (2019) from a village named Kohraura in Uttar Pradesh. The collected mahua flowers were sun - dried for a period of three days (8 hours/day) under hygienic condition. Then they are collected and stored in an airtight container and brought to Chennai for further experimental study.

B. Preparation of Aqueous Extract

In the present study, aqueous extract of dried mahua flowers was used as a standard solvent to carry out the experimental analysis due to predominant use of the solvent at the household stage. The extract was prepared using heating method. To prepare the aqueous extract, 5 g of the dried flowers were weighed and powdered coarsely using mortar - pestle. Then the powder was mixed to 100 ml of distilled water in a conical flask. The mouth of the conical flask was closed tightly using cotton and aluminum foil in order to avoid the vapor to escape. This setup was placed on water bath for a period of approximately 30 minutes. Later, it was cooled, filtered, and transferred to appropriately labeled bottle and stored in refrigerator at 4^0 C.

C. Estimation of Phytochemicals

The qualitative and quantitative phytochemicals analysis and antioxidant study of dried mahua flowers was carried out at Apex Biotechnology training and Research Institute located at Ekkattuthangal, Chennai, Tamil Nadu.

1) Qualitative analysis of Phytochemical

Methods employed for the qualitative analysis of the phytochemicals in present study are,

a) Alkaloids

Mayer's test: A drop of Mayer's reagent was added by the side of the test tube to a few ml of the filtrates. Appearance of creamy or white precipitate indicates the positive result (Velavan, 2015).

b) Anthraquinones

Borntrager's test: To 3ml of extract, dilute sulfuric acid was added and then it was boiled, filtered and cooled. An equal volume of benzene was added to the filtrate and was shaken well to separate the organic layer. To the organic layer equal volume of 10% ammonia solution was added. The ammonia layer turned pink/red/violet colour showing the presence of anthraquinone (Rufia, Isah and Isyaka, 2016).

c) Flavonoids

Alkaline reagent test: To 1 ml of the extract, 2 ml of the 10 % NaOH solution was added resulting in the formation of intense yellow color which disappears on the addition of dilute hydrochloric acid. The disappearance of intense yellow coloration indicates the presence of flavonoids in sample (Velavan, 2015).

d) Total phenols

Ferric chloride test: To 1 ml of the extract, few drops of ammonia was added followed by the few drops of 10% aqueous ferric chloride. The appearance of reddish- brown coloration showed the presence of phenolic compounds (Manupati, et.al., 2014).

e) Coumarins

3 ml of alcoholic sodium hydroxide (10% sodium hydroxide in methanol) was added to 2 ml of aqueous extract. The formation of yellow color indicates the presence of coumarins (Rufia, et.al., 2016).

f) Terpenoids

Salkowski's test: To the extract, 2 ml chloroform was added followed by the 3 ml of concentrated sulphuric acid around the layer of the test tube. Appearance of reddish- brown at the interface shows the presence of terpenoids (Velavan, 2015).

g) Cardiac glycosides

Keller- Killani test: To 1 ml of the extract, 1 ml of glacial acetic acid was added followed by one drop of ferric chloride solution. This was under-laid with 1 ml of concentrated sulfuric acid. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides (Velavan, 2015).

h) Steroids

Liebermann - Burchard test: To 1 ml of the extract, 3 ml of acetic anhydride and few drops of glacial acetic acid were added. It was cooled and a drop of concentrated sulphuric acid was added. Appearance of blue/bluish green colouration indicates the steroidal ring (Manupati, et.al., 2014).

i) Saponins

Froth test: About 1 ml of the extract was diluted to 5 ml with distilled water in a test tube and shaken well for 5 minutes. The formation of foam in the upper part of test tube indicates the presence saponins in the sample (Velavan, 2015).

j) Tannins

Ferric chloride test: To 1 ml of the extract, 4 ml of 1% ferric chloride was added. The formation of green/blue-black/ blue-green precipitate indicates the presence of tannins in the sample (Velavan, 2015).

2) Quantitative Estimation Of Phytochemicals

Determination of Total Alkaloids Content

Bromocresol Green Method

A part of the sample was dissolved in 2 N HCl (Hydrochloric acid) and then filtered. One ml of this solution was washed with 10 ml chloroform thrice in a separatory funnel. The pH was neutralized with 0.1 N NaOH. Then, 5 ml of each of BCG solution and phosphate buffer were added. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance was measured at 470 nm against blank. Atropine is used as standard. The total alkaloids level is calculated and represented in atropine equivalent (John, et.al., 2014).

Determination of Total Flavonoid Content

Aluminium Chloride Method

Aqueous extract was mixed with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. After the 40 minutes of incubation at the room temperature, the absorbance for the mixture was determined spectrophotometrically at 415 nm. Quercetin was chosen as a standard and the total flavonoid content was expressed as milligram quercetin equivalents per g of dry extracts (Velavan, 2015).

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Determination of Total Phenolic Content

Folin-Ciocalteau Phenol Reagent Method

To the 200 µL of the extract in a screw capped test tube, 1.0 ml of Folin-Ciocalteau reagent (1:1 with water) and 1.0 ml of sodium carbonate (7.5%) were added. The tubes were vortexed incubated for 2 hours and the absorbance was read at 726 nm using a spectrophotometer. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram dry extract (Velavan, 2015).

3) Estimation Of Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow et al. (1997). Two ml of 6×10^{-5} M methanolic solution of DPPH was added to 50 µl of a methanolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$IP = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidants at t = 16 min.

4) Nutrition Estimation Of Dried Mahua Flowers

The various nutrients estimated and the method employed for estimation has been presented in the table below:

S.No.	Nutrient	Method
1	Moisture	IS 4333 (Part 2):2017 - ISO 712:2009
2	Ash	IS 13854:1994 - ISO 1575:1987
3	Protein	IS 7219:1973 RA.2010
4	Fat	AOAC 20th edition 2016.920.85
5	Dietary fibre	IS 10226 (Part II) 1982
6	Carbohydrates	AOAC 20th edition 2016 986.25
7	Energy	SOP/FOOD/114
8	Iron	AOAC 999.11 20th edition 2016
9	Calcium	IS 15121:2002 RA.2013

III. RESULTS AND DISCUSSION

1) Qualitative screening of phytochemicals in dried *Madhuca longifolia (*mahua) flowers

S. No.	Phytochemicals	Inference
1.	Alkaloids	Present
2.	Anthraquinones	Absent
3.	Flavonoids	Present
4.	Total phenols	Present
5.	Coumarins	Present
6.	Terpenoids	Present
7.	Cardiac glycosides	Present
8.	Saponins	Present
9.	Steroids	Present
10.	Tannins	Absent

The major chemical components in Mahua are quercetin, β - amyrin decanate, betullic acid, tannins, β-amyrin, β– amyrin acetate, stigma sterol and β- amyrin cinnamate (Sharma, et.al., 2013)**.** Earlier studies on *Madhuca longifolia* included characterization of

sapogenins, carbohydrates, triterpenoids, steroids, saponins, flavonoids, and glycosides (Ramadan, et.al., 2016). The present study revealed that among the different screened phytochemicals, alkaloids, flavonoids, total phenols, coumarins, terpenoids, cardiac glycosides, saponins and steroids showed positive result while anthraquinones and tannins showed negative result in aqueous extract of dried mahua flowers. The steroidal compounds are found to possess importance in pharmacy due to their association with compounds such as sex hormones (Annalakshmi, et.al., 2012)**.** The present study is in conjunction with the study conducted by Annalakshmi, et.al., (2012) which reported the presence of various phytoconstituents like alkaloids, phenolic compounds, flavonoids, protein and amino acids, tannins, sterols and saponins in mahua flowers.

Fresh mahua flowers examined for bioactive components using different solvents such as aqueous, ether, acetone and methanol, disclosed the presence of carbohydrates, proteins, flavonoids and tannins in all four extracts while alkaloids were present in aqueous and ether extract, saponins were present only in methanolic extract. Sterol was present in ether, acetone and methanol extract whereas lipid was found to be present in aqueous, ether and methanolic extracts (Sinha, et.al., 2017).

2) Quantitative estimation of phytochemicals in dried *Madhuca longifolia* (mahua) flowers

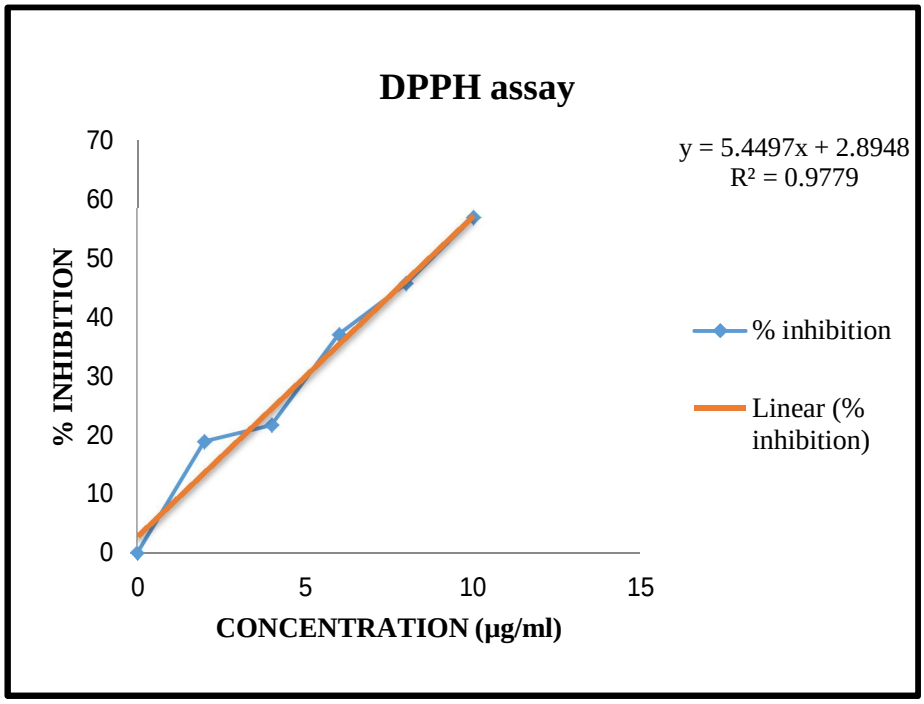
S. No.	**Phytochemicals**	**Content (per g dry extract)**
1.	Total alkaloids content	12.28 mg AE
2.	Total phenol content	107.00 mg GAE
3.	Total Flavonoids content	1.217 mg QE
4.	Total saponins	0.163 mg DE

* AE : Atropine Equivalent; GAE : Gallic Acid Equivalent; QE : Quercetin Equivalent; DE : Diosgenin Equivalent.

The quantitative composition estimated for the selected phytochemicals in the present study, revealed that dried mahua flowers contain around 12.28 mg alkaloids of atropine equivalents per grams of dry extract. An analogous study conducted by Mir, et.al., (2016), on the quantitative estimation of phytochemicals in the flowers of *Crocus sativa* which is also from the Sapotaceae family reported that the alkaloid content in methanol and water extracts of the flowers were found to be 6.4 and 2.4 mg/g of dry extract respectively. The flavonoids concentration of aqueous extract of dried mahua flowers in the present study was found to be very minimal. the total phenol concentration of aqueous extract of dried mahua flowers found in the present study was 107 mg total phenol of Gallic acid equivalent per grams of dry extract. A similar study performed on the phenol content of mahua in combination with guava as a nutra-beverage by Soni and Dey (2013) estimated the total phenolic content of the product to be 171.83 ± 5.21 mg Gallic acid equivalent per liter.

3) Antioxidant activity using DPPH assay

S. No.	Concentrations (µg/ml)	Absorbance level	DPPH free radical inhibition %	IC_{50} (Maximum inhibitory concentration)
1.	0	0.648	0.00	8.64 µg/ml
2.	2	0.525	18.98	
3.	4	0.507	21.76	
4.	6	0.407	37.19	
5.	8	0.351	45.83	
6.	10	0.278	57.10	



DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures the ability of a compound to act as free radical scavenger or hydrogen donor (Boly, et.al., 2016). Dose dependent activity of aqueous extract was observed in the present study. The extract exhibits maximum inhibitory activity (IC_{50}) at the concentration of 8.64µg/ml against DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical activity. It has been discovered that with the decrease in DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) value, there is an increase in the radical scavenging activity or antioxidant activity. Therefore, present study indicates towards the superior antioxidant activity of dried mahua flowers extract. A similar study dealt on the antioxidant activity of different medicinal plants using DPPH reported that the methanolic extract of *M. longifolia* (62.45 mg/L) possessed stronger DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging potential when compared to other medicinal plants analysed (Arun, et.al., 2017). A parallel study carried out on the nutra-beverage potential of mahua flowers showed the highest antioxidant activity of 96.5 % and 89.6 % with ABTS (2,2'-Azino-Bis-3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assays respectively. It has been stated that the mahua-guava product showed higher degree of protection against lipid peroxidation (Soni & Dey, 2013).

4) Nutrition composition of Dried mahua flowers

S.No.	Nutrients	Quantity/100g of dry flowers
1	Moisture	15.27 g
2	Ash	9.17 g
3	Protein	3.14 g
4	Fat	4.02 g
5	Fibre	19.61 g
6	Carbohydrate (by difference)	48.79 g
7	Energy	243.9 Kcal
8	Calcium	1.24 mg
9	Iron	8.064 mg

It was observed in the present study that apart from being rich source of phytochemical and antioxidant property mahua flowers also contain good amount of nutrients like iron, fibre, carbohydrates, protein and fat.

A similar study highlighting the medicinal and commercial potential of *Madhuca indica* by Meena and Meena (2016) stated the nutritional contents of *Madhuca* flower are 54.24% total invert sugar, 50.62% Reducing sugar, 3.43% Cane sugar, 54.06% total sugar, 19.8% Moisture, 6.37% Protein, 0.5% Fat, 4.36% ash, 8% calcium and 2% phosphorous. Another study conducted by Patel et al., (2012) reported that mahua flower contains carotene, ascorbic acid, thiamine, riboflavin, niacin, folic acid, biotin and inositol. The nutrition composition of mahua flowers have been found to deteriorate with the storage duration (Akshatha, et.al., 2013). A decrease in 0.05-1.5% vitamin C content with the increase in storage duration which could possibly be due to ascorbic acid degradation (approx. 88%) by ascorbic acid oxidase. No considerable effect was observed in B vitamins (Yadav, 2013).

IV. CONCLUSION

The present study concludes that dried mahua flowers contain phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, cardiac glycosides, steroids, and coumarins. The total alkaloids and total phenol concentration determined in the dried mahua flowers enhances its pharmacological importance and utilization. The significant antioxidant activity of the flowers against DPPH can be attributed to the high content of alkaloids and total phenol. Presence of various phytochemicals and nutrient make the flowers an efficient source for the utilization in the food industry targeting the community health. Future research studies can also be carried out towards quantitative determination of various other phytochemicals present and also its reliable utilization in the treatment of prevailing diseases via., in- vitro and in-vivo studies.

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Author declare no conflict of interest towards the present research work.

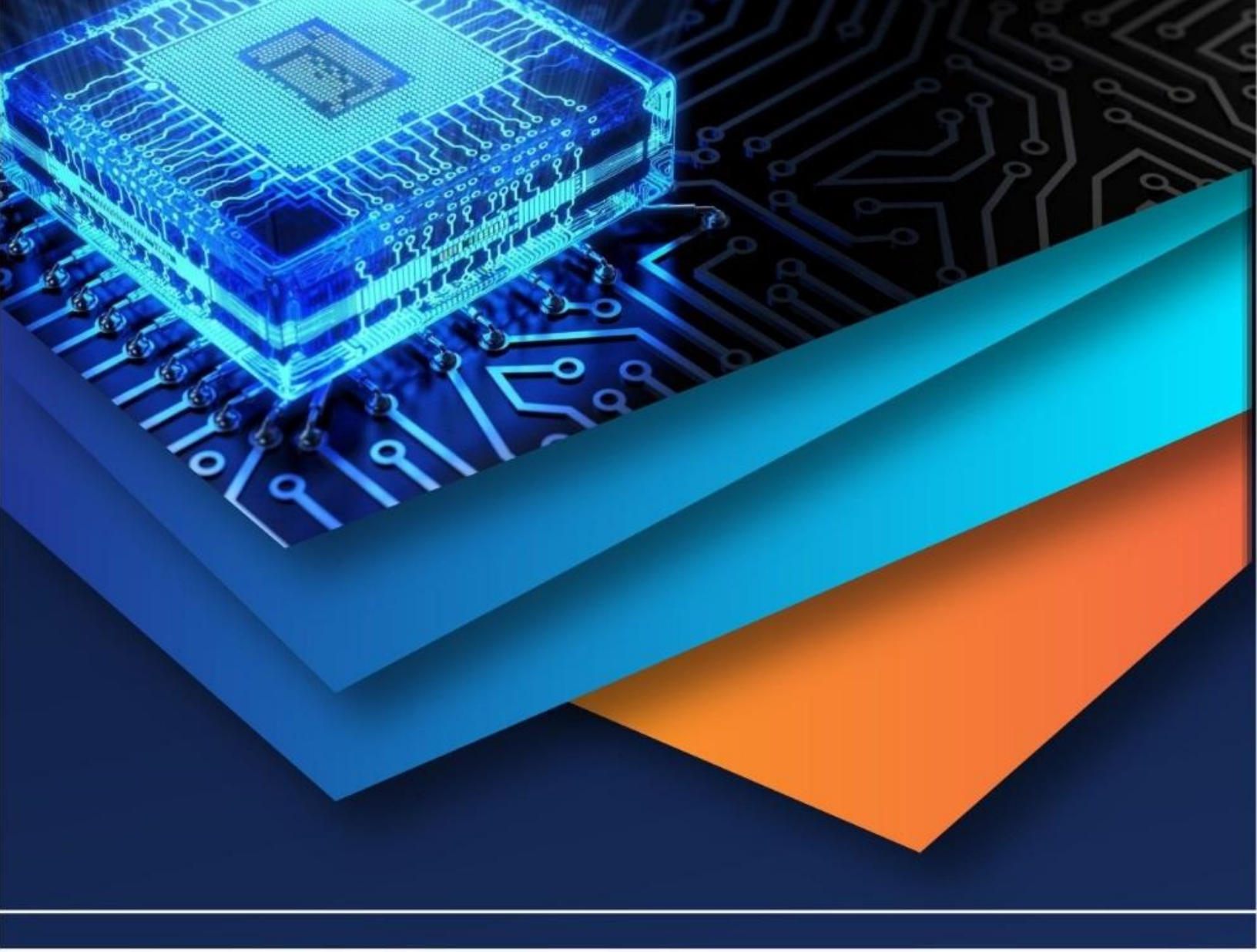
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