iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH
IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 Issue: VII Month of publication: July 2025

DOI: https://doi.org/10.22214/ijraset.2025.73109

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue VII July 2025- Available at www.ijraset.com

An Overview of Berries' Bioactive Components

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I. INTRODUCTION

Fruits are high in nutritive elements such as vitamins, minerals, carbohydrates, and essential oils; therefore their consumption has risen in recent years. Fruit juices include bioactive chemicals that have anticancer, antimutagenic, antibacterial, anti-inflammatory, and anti-neurodegenerative activities. Berries are the most commonly consumed fruit since they are delicious and give several health advantages. Berries from the Rosaceae (strawberry, raspberry, and blackberry), Ericaceae (blueberry), and Vitaceae (black and green grapes) families are high in BAC (bioactive compounds)(Skrovankova et al., 2015).

In Europe, America, and Australia, berries are widely farmed and consumed. (Yang et al., 2020).They can be eaten raw, frozen, or made into wines, juices, and jams(Siracusa et al., 2019). The most often consumed berries are blackberry (Rubus spp.), blueberry (Vacciniumcorymbosum), red raspberry (Rubusidaeus), and strawberry (Fragaria spp.)(Luíset al.,2018) .Berryfruits have recently piqued the interest of academics all over the world due to its high content and diverse spectrum of health-promoting phenolic chemicals(Stoner et al., 2015).Phenolic molecules stand out among the antioxidant components present in fruits and vegetables. They are found in fruits, vegetables, leaves, nuts, seeds, and flowers and occur naturally in plants as secondary metabolites. They are an important part of the human diet and have been added to several medicine compositions on purpose(WU et al., 2004).

The most powerful antioxidants in food are phenolic chemicals, which provide a wide range of health benefits(Cox et al., 2005). Plant products antioxidant action is mostly related to phenolic chemicals(Chua et al., 2008). As a result, the therapeutic efficacy of a diet rich in fruits with high levels of natural antioxidants is important(Sokol-Letowska et al., 2007), and their ability to neutralize free radicals (Stratil et al., 2007).Flavonoids, tannins, and phenolic acids are abundant in blueberries, as they are in most berries. Many studies have shown that the presence of such bioactive substances, particularly anthocyanins, in the blueberry offers a number of health benefits(Heinonen et al., 1998, Smith et al., 2000; Seeram, 2008).Organic matter makes up the majority of agro-industrial waste, which is often high in sugars and fiber. These wastes have a great nutritional value, but when produced in big amounts, they pose an environmental threat. Furthermore, bioactive compounds with health-promoting qualities and possible technological applications, such as antioxidants and even food dyes, may be present in these residues(Lee &Wrolstad., 2004).Thus, these wastes represent a potentially useful resource tobe explored.

When oxidative species exceed the organism's anti-oxidative defenses, resulting in oxidative stress, free radicals and other reactive species can cause oxidation and biomolecular damage. This is linked to the onset of diseases such as cancer, cardiovascular disease, neurological illnesses, diabetes, and inflammation as people age (DUFFY et al., 2007; MATEOS; BRAVO, 2007; LU; FINKEL, 2008).

Plant metabolism is known to be separated into primary and secondary metabolism. The fundamental metabolism produces the components that are universal to all living things and are required for cell maintenance (lipids, proteins, carbohydrates, and nucleic acids). Secondary metabolism, on the other hand, produces chemicals that come from many biosynthetic pathways and are confined to specific types of organisms. One of the largest and most widely distributed families of secondary metabolites in plants is phenolic compounds. (Scalbert et al., 2000).

Biogenetically, phenolic compounds are generated through two metabolic pathways: the shikimic acid pathway, which produces phenylpropanoids primarily, and the acetic acid process, which produces the simple phenol as the principal result. The majority of plants produce phenolic chemicals.Thephenylpropanoidpathway is involved. When both paths are combined, the result is Flavonoids, the most abundant category of phenolic chemicals in nature, are formed(Sánchez-Moreno, 2002; Hollman, 2001).

Phenolic molecules are necessary for cellular metabolism and function.They play a role in a variety of functions in plants, including sensory characteristics (colour, scent, taste, and astringency), structure, pollination, pest and predator resistance, seed germinative processes after harvesting, growth, development, and reproduction (Tomás-Barberán,2001;Espín, 2001).

The antioxidant activity of food phenolic compounds is of nutritional relevance because it has been linked to the potentiation of human health-promoting benefits through disease prevention (Lampe, 1999).Additionally, due to their pharmacological properties, these chemicals may be employed for medicinal purposes in some situations (Percival, 1998). Because of its toxicity, many phenolic compounds with low molecular weight, such as thymol, are utilised as antiseptics in medicine (Harborne, 1980).

II. NUTRIENT COMPOSITION

Berries have a high concentration of important vitamins, dietary fibre, and minerals. Berries have high sugar content, yet are low in calories and fats. Vitamin C, dietary fibre, potassium, and folates are all found in raspberries, blackberries, and blackcurrants. Vitamin C values in these berries range from 9.7 to 60 mg/100 g, with blueberries having the lowest and strawberries having the highest. Berries including strawberries, blackberries, and raspberries are high in folate (vitamin B9) and potassium. Blackberries and blueberries are high in vitamin K, whereas cranberries are strong in vitamin E. Beta-carotene, lutein, and zeaxanthin are abundant in blackberries. Among these berries, blackcurrants have the highest quantities of calcium, iron, phosphorus, and potassium. (Haytowitz et al., 2018).

Table 1. Nutrient composition (value per 100 g fresh weight)
(Haytowitz, D.B. and Pehrsson, P.R., 2018)

Nutrient	Strawberry	Blackberry	Raspberry	Cranberry	Blueberry	Blackcurrant
Water (g)	90.95	88.15	85.75	87.32	84.21	83.95
Energy (kcal)	32	43	52	46	57	56
Protein (g)	0.67	1.39	1.2	0.46	0.74	1.4
Total lipid (fat) (g)	0.3	0.49	0.65	0.13	0.33	0.2
Carbohydrate (g)	7.68	9.61	11.94	11.97	14.49	13.8
Fiber, total dietary (g)	2	5.3	6.5	3.6	2.4	4.3
Sugars, total (g)	4.89	4.88	4.42	4.27	9.96	7.37
Calcium, Ca (mg)	16	29	25	8	6	33
Iron, Fe (mg)	0.41	0.62	0.69	0.23	0.28	1
Magnesium, Mg (mg)	13	20	22	6	6	13
Phosphorus, P (mg)	24	22	29	11	12	44
Potassium, K (mg)	153	162	151	80	77	275
Sodium, Na (mg)	1	1	1	2	1	1
Zinc, Zn (mg)	0.14	0.53	0.42	0.09	0.16	0.23
Copper, Cu (mg)	0.048	0.165	0.09	0.056	0.057	0.107
Selenium, Se (µg)	0.4	0.4	0.2	0.1	0.1	0.6
Vitamin C (mg)	58.8	21	26.2	14	9.7	41
Thiamin (mg)	0.024	0.02	0.032	0.012	0.037	0.04
Riboflavin (mg)	0.022	0.026	0.038	0.02	0.041	0.05
Niacin (mg)	0.386	0.646	0.598	0.101	0.418	0.1
Vitamin B6 (mg)	0.047	0.03	0.055	0.057	0.052	0.07
Folate, total (µg)	24	25	21	1	6	8
Vitamin A (µg)	1	11	2	3	3	2
Carotene, beta (µg)	7	128	12	38	32	25
Carotene, alpha (µg)	0	0	16	0	0	0
Lutein + zeaxanthin (µg)	26	118	136	91	80	47
Vitamin E (mg)	0.29	1.17	0.87	1.32	0.57	0.1
Vitamin K (phylloquinone) (µg)	2.2	19.8	7.8	5	19.3	11

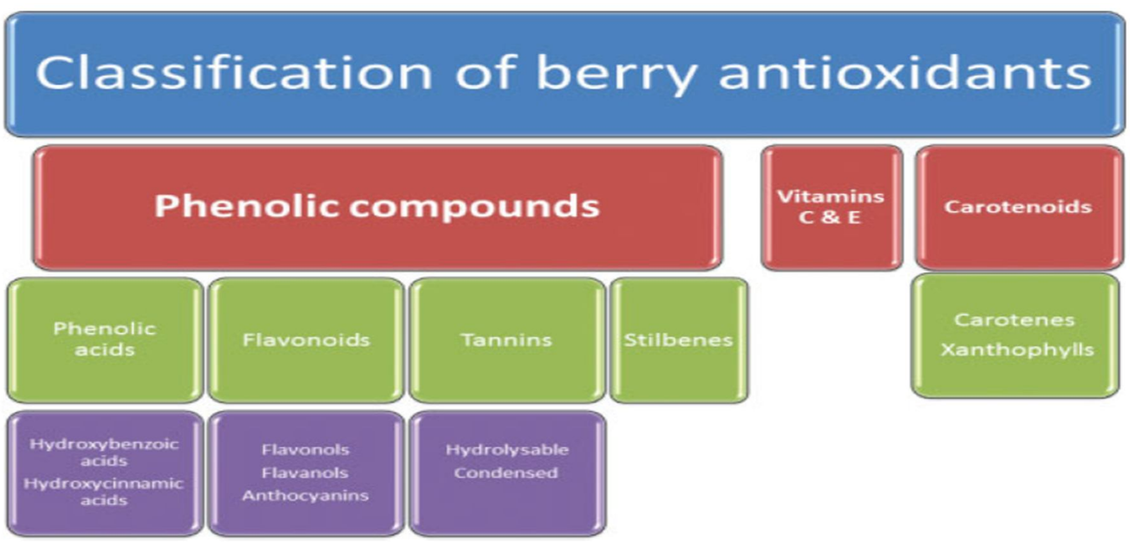

Fig.1 Classes of Polyphenols
(Manganaris et al., 2014)

A. Classes of Polyphenols

A common intermediary, phenylalanine, or a similar precursor, shikimic acid, is the source of all plant phenolic compounds. They are most commonly found conjugated, with one or more sugar residues attached to hydroxyl groups, but direct connections of the sugar (polysaccharide or monosaccharide) to an aromatic carbon are also possible. It is also typical to associate with other substances such as carboxylic and organic acids, amines, lipids, and other phenols(Kondratyuket al., 2004).

Berry fruits have a high concentration of phenolic compounds and a diverse range of them. They are represented by phenolic acids (benzoic and cinnamic acid derivatives), tannins, stilbenes, and flavonoids such as anthocyanins, flavonols, and flavanols, which differ in structure and molecular weight (catechins)(Shahidi F et al., 2004,Puupponen-Pimiäet al., 2005, Cieślik et al., 2006).Phenolics are a broad group of secondary metabolites that consist of one or more aromatic rings with varying degrees of hydroxylation, methoxylation, and glycosylation, and contribute to the colour, astringency, and bitterness of fruits (Crozier et al.,2006).

B. Phenolic Acids

Benzoic acids and cinnamic acids, as well as their derivatives, are two types of phenolic acids. The simplest phenolic acids found in nature are benzoic acids, which have seven carbon atoms (C6-C1). Cinnamic acids have nine carbon atoms (C6-C3), while the ones found in vegetables most usually have seven. The presence of a benzenic ring, a carboxylic group, and one or more hydroxyl and/or methoxyl groups in the molecule distinguishes these compounds. Phenolic acids may account for one-third of all phenolic chemicals consumed by humans(Yanget al., 2001).

Although other characteristics contribute to the antioxidant activity of phenolic acids and their esters, this activity is usually determined by the number of hydroxyl groups found in the molecule of these substances and their esters, especially hydroxybenzoic acid, hydroxycinnamic acid, caffeic acid, and chlorogenic acid, and although other characteristics contribute to the antioxidant activity of phenolic acids and their esters, this activity is usually determined by the number of hydroxyl groups found in the molecule of these substances and their Cinnamic acids that have been hydroxylated are more efficient than benzoic acids in general.(Sánchez-Moreno,2002).

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Cinnamic acids have nine carbon atoms (C6-C3), however the most common is cinnamic acid (C6-C3).Seven is a number that is commonly encountered in vegetables. These chemicals are distinguished by the fact that they have one or more hydroxyl and/or methoxyl groups, a benzenic ring, a carboxylic group, and one or more hydroxyl and/or methoxylgroups in the molecule (Yang et al., 2001).

Cinnamic acids are rarely found in plants in their natural state. They're usually in the form of esters, with a cyclic alcohol-acid like quinic acid to make isochlorogenic acid, neochlorogenic acid, criptochlorogenic acid, and chlorogenic acid, as well as a caffeoyl ester, which is the most important combination(Bravo,1998).

Phenolic acids may account for one-third of all phenolic chemicals consumed by humans.(Yang et al., 2001).Although other characteristics contribute to the antioxidant activity of phenolic acids and their esters, this activity is usually determined by the number of hydroxyl groups found in the molecule of these substances and their esters, especially hydroxybenzoic acid, hydroxycinnamic acid, caffeic acid, and chlorogenic acid. Cinnamic acids that have been hydroxylated are often more effective than benzoic acids.
(Sánchez-Moreno,2002).

C. Flavonoids

Variations in the hydroxylation pattern and oxidation state of the core pyran ring give birth to a diverse spectrum of chemicals, including flavanols, anthocyanidins, anthocyanins, isoflavones, flavones, flavonols, flavanones, and flavanonols. The existence or absence of a double bond between C2 and C3, as well as the production of a carbonyl group by C4, determine the hydroxylation pattern and oxidation state. Flavones, flavonols, flavanones, and flavanonols are the most abundant subgroup of polyphenols, accounting for the bulk of flavonoid compounds (D'Archivio, M et al.2007). Tannins can be separated into two types: hydrolysable tannins and condensed tannins, which are found in complexes with alkaloids, polysaccharides, and proteins (Crozier, A. et al ,2006).

Table 2: Major flavonoid subclasses

Subclass	C2–C3 Bond group	C3 Hydroxyl group	C4 Carbonyl group	Ring B Position	Glycosylation	Notable Examples	Natural Sources	Refrences
Flavones	Present	Absent	Present	C2	Common	Apigenin, Luteolin	Parsley, celery, chamomile	(deLacerda de Oliveira, L et al.2014),(Tsao, 2010).
Flavonols	Present	Present	Present	C2	Common	Quercetin, Kaempferol	Onions, kale, apples	
Flavanones	Absent	Absent	Present	C2	Common	Hesperidin, Naringenin	Citrus fruits (oranges, lemons)	(Reis-Giada, 2014).(de Lacerda de Oliveira et al. 2014).
Flavanonols	Absent	Present	Present	C2	Less Common	Dihydrokaempferol	Tea, grapes, certain fruits	
Anthocyanidins	Present	Variable	Present	C2	Absent	Cyanidin, Delphinidin	Red cabbage, berries	(Reis-Giada, 2014). (Crozier, A. et al ,2006), (Ozcan, T et al ,2014),(He and Giusti, 2009; Lee et al., 2015)
Anthocyanins	Present	Variable	Present	C2	Present	Pelargonidin-3-glucoside	Berries, red grapes, purple corn	
Isoflavones	Present	Absent	Present	C3	Common	Genistein, Daidzein	Soybeans, legumes	(Lattanzio, V. 2013), (Reis-Giada, M.d.L.et al , 2014).

D. Stilbenes

A two-carbon methylene bridge connects the two phenyl moieties in stilbenes. Stilbenes are found in very small amounts in the human diet. The majority of plant stilbenes are antifungal phytoalexins, which are chemicals produced solely in reaction to infection or harm. Resveratrol (3,4',5-trihydroxystilbene), found primarily in grapes, is a well-studied naturally occurring polyphenol stilbene. Red wine is made from grapes and contains resveratrol in large quantities(Crozier, A. et al.,2006).

E. Lignans

Lignans are diphenolic chemicals with a 2,3-dibenzylbutane structure created by dimerization of two cinnamic acid residues. Phytoestrogens include lignans like secoisolariciresinol. Linseed is the most abundant dietary source, with up to 3.7 g/kg dry weight of secoisolariciresinol and modest levels of matairesinol(AdlercreutzH,et al.,1997).

F. Tannins

Tannins are phenolic compounds with molecular weights ranging from 500 to 3000 D that fall into two categories: hydrolysable tannins and non-hydrolysable or condensed tannins(Chung, K. T et al.,1986)Phlorotannins are a third type of tannin found only in brown seaweeds and are not typically taken by humans(Ragan, M. A.et al., 1986). Gallotannin and ellagitannin, respectively, are hydrolysable tannins having a glucose or polyhydric alcohol core partially or totally esterified with gallic acid or hexahydroxydiphenic acid(Okuda, T.et al., 1995).Acids, bases, and enzymes can easily hydrolyze these metabolites. They can, however, be oxidatively condensed to other galoil and hexahydroxydiphenic molecules, resulting in high-molecular-weight polymers. Tannic acid, the most well-known hydrolysable tannin, is a gallotannin composed of a pentagalloyl glucose molecule that can also be esterified with another five units of gallic acid (Bravo, L. 1998). Despite the fact that tannins have a lower antioxidant activity than flavonoids, current study has demonstrated that the degree of polymerization of these compounds is connected to their antioxidant activity. This activity can be up to fifteen to thirty times greater than that of simple phenols in condensed tannins and hydrolysable (ellagitannins) of high molecular weight(Sánchez-Moreno, C.,2002).

G. Carotenoids

Carotenoids are found in modest amounts in berry fruits (Heinonen MI, et al.
1989, .Razungles A, et al,1989, Marinova D, et al.,2007). Chokeberry is one of the most abundant sources of carotenoids, with an average amount of 48.6 mg/kg fresh weight. Lycopene, -carotene, -carotene, -cryptoxanthin, lutein, 5,6-epoxylutein, trans-violaxanthin, cis-violaxanthin, and neoxanthin are all found in chokeberry fruits (Razungles A, et al.,1989).

H. Vitamin C

One of the most important water-soluble vitamins is ascorbic acid. It can be found in a variety of fresh fruits and vegetables. Ascorbic acid is produced by most plants and animals from D-glucose or D-galactose. Humans, on the other hand, are unable to produce ascorbic acid due to a lack of the enzyme L-gulonolactone oxidase. Vitamin C is also known as L-ascorbic acid. 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol is the chemical name. The most common dietary forms of vitamin C are L-ascorbic acid and dehydroascorbic acid. (Naidu KA., 2003).

I. Extraction

Five grams of berry slurry were extracted with 20 mL of 70% ethanol using a modified version of Gu et al. (2019). Samples were homogenized at 10,000 rpm for 30 s (Ultra-Turrax T25), then shaken at 120 rpm for 12 h at 4 °C. After centrifugation at 5,000 rpm for 15 min at 4 °C (Hettich ROTINA 380R), the supernatant was filtered through a 0.45 µm syringe filter (Thermo Fisher) prior to analysis by LC-ESI-QTOF-MS/MS and HPLC-PDA. Six grams of berry and seed samples were extracted with 15 mL of 80% aqueous methanol. The mixture was incubated for 2–3 h at 70 °C with orbital shaking, then centrifuged at 2,500 rpm for 15 min. The resulting filtrate was used to assess total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and antimicrobial activity via a Buchner funnel filtration step (Patel, N *et al.*,2021). Ground blueberry samples (2.00 g) were extracted in 60 mL methanol using an ultrasonic water bath (USC-1400 Unique) at 20 °C for 15 min. The mixture was centrifuged at 2,000 g for 10 min, and the supernatant was stored in an amber flask for total phenolic (TP) and antioxidant (AA) assays. Separately, ground blueberry (1.50 g) was extracted in 15 mL acidified methanol (0.1% HCl) under ultrasonication (20 °C, 30 min), followed by centrifugation at 2,000 g for 10 min to collect the supernatant for total anthocyanin (TA) analysis.(Rodrigues, E et al.,2011).

J. Determination of Total Phenolic Content (TPC) of berries

Using distilled water, various concentrations / dilutions of extracts was generated (1:2, 1:5, 1:10, undiluted). The test tube was filled with 1 ml of extract from each concentration. After that, 0.5 mL of folin-ciocalteu reagent was added to each concentration, and the mixture was incubated in the dark for 10 minutes. Following incubation, 2 mL of 20% sodium carbonate was added to the mixture, which was then incubated in the dark for 30 minutes. The absorbance was then measured using a colorimeter at 700 nm against a blank. The slope of the gallic acid standard calibration curve was used to calculate the concentration of total phenolic content in the extracts. The total phenolic content in g/ml present in the sample was then determined using the average of values(Patel N *et al.*2021).The following formula was used to calculate the concentration of phenolic content:

Concentration (µg/ml) = Absorbance at 700 nm / slope of the calibration curve

The Folin-Ciocalteau technique (G.J. McDougall *et al*., 2005) was used to determine total phenolic compounds. 235L water, 5L sample (or solvent in the blank), 15L Folin-reagent Ciocalteau's (Fluka®), and 45L saturated Na_2CO_3 were added to each well of a microplate. The microplate was incubated at 40°C for 30 minutes, and the absorbance was measured.The wavelength was measured at 765 nm. The standard was gallic acid, and the results were given in mg of gallic acid equivalents (GAE in milligrammes).

Phosphowolframic acid and phosphomolybdic acid together the Folin-Ciocalteu method was used to quantify total phenolic compounds using acid in basic dissolution. The blue color's absorbance was measured at 765 nm. in a spectrophotometer (Varian Cary 50-Bio, Victoria, BC) at 20°C Australia). 40 litres of each berry were used in the study. 500 mL Folin-Ciocalteu reagent was used after that. After that, 2mL of sodium carbonate (20%) was added, and finished with 10mL of miliQwater. After shaking, the wavelength was measured at 765 nm.Finally, absorbance was calculated. (Marhuenda, J. et al,2015)

K. Determination of total anthocyanins (TA)

Using a spectrophotometer (Hewlett-Packard 8452 A Spectrophotometer) and the Folin-Ciocalteau technique, the total phenolic content of each extract was determined (SINGLETON; ROSSI, 1965). The absorbance was measured at 765 nm, and the results were reported as mg.100 g–1 gallic acid equivalent in fresh weight (GAE).

Using a 1 percent HCl in methanol solution, the anthocyanins were extracted completely from a sample of about 2 g. After that, the solution was filtered and vacuum concentrated (at a temperature of 38 °C) in a rotary evaporator (Fisatom, model 801/802, SPaulo, SP)(Brazilian) (Francis, 1982). The crude extract was concentrated.The volume was then transferred to a 25 mL flask and completed withthe methanol solution that has been acidified This solution in a 1 mL aliquotwas taken out, dried with nitrogen gas (N2), and kept frozen(T –18 °C) till the time of analysis. This extract has been dried in N2 (or 1 mL of water)chromatographic grade dilution of previously filtered juicemethanol (unique, model) homogenised in an ultrasonic bathIndaiatuba, SP, Brazil) and filtered into a vial via USC 1400a polyethylene-based material.
The anthocyanins were measured using High Performance Liquid Chromatography. HPLC was used to identify the samples, which were then compared to acceptable standards. The solvents that were employed in the the HPLC separations were of chromatographic quality.Millipore's vacuum filtration equipment was used to filter the water. Prior to use, a 0.45 mm membrane for organic solvents (Millipore, Brazil,Barueri, SP).

L. Determination of Total Flavonoid Content (TFC) of berries

Extracts were made in various concentrations/dilutions (1:2, 1:5, 1:10, undiluted). 1 ml of each concentration's extract was put to the test tube along with 1 ml of distilled water, and then 0.2 ml of 5 percent sodium nitrate was added to the mixture. After that, the mixture is incubated for 6 minutes at room temperature. After that, 0.3 mL of a 10% aluminium chloride solution was added to the mixture, which was then incubated at room temperature for 5 minutes. Then, 0.4 ml of 1M sodium hydroxide was added to the mixture, thoroughly mixed, and the absorbance at 510 nm was measured against a blank. Total flavonoid content concentrations were estimated using the slope of the standard quercetin (Patel N *et al.*2021). The following formula was used to determine the concentration:
Concentration (µg/ml) = Absorbance at 510 nm / slope of the calibration curve

M. Determination of Antioxidant Activity

A FRAP (ferric reducing antioxidant potential) experiment was used to assess the antioxidant activity of berries and seed extracts. It's a quick and easy way to determine the antioxidant potential of a substance. 1 ml extract, 1 ml 0.2 M phosphate buffer, and 1 ml 1 percent potassium ferricyanide were combined together. After that, the mixture was incubated for 20 minutes at 50 degrees Celsius. Following the incubation period, 1 mL of 10% trichloroacetic acid was added to the mixture. The mixture was then centrifuged at 3000 rpm for 10 minutes. After that, the upper layer (1 ml) was mixed with 0.5 ml of 0.1 percent ferric chloride. At 700 nm, the absorbance was measured. The concentration of ferrous ammonium sulphate was determined using a standard ferrous ammonium sulphate calibration curve. (Patel N *et al.*2021).
Concentration (µg/ml) = Absorbance at 700 nm / slope of the calibration curve
To determine the concentration of total antioxidant, the ferric reducing antioxidant power (FRAP) assay was utilised. When the TPTZ-Fe3+ complex is reduced to the TPTZ-Fe2+ form (2,4,6-tripyridyl-s-triazine) in the presence of antioxidants, the result is a strong blue colour.All reductants with half-reaction reduction potentials greater than Fe3+/Fe2+ cause the reduction to occur quickly (Pellegrini et al., 2003).
The FRAP experiment was carried out using FRAP reagent (1 mM 2,4,6-tripyridyl-2-triazine [TPTZ]) and 20 mM ferric chloride in 0.25 M sodium acetate, pH 3.6. 100 mL extract was added to 1 mL FRAP reagent and carefully mixed. The absorbance at 593 nm was measured against a water blank after standing at room temperature (20 °C) for 4 minutes. Calibration was performed using freshly made ammonium ferrous sulphate and a standard curve (100–600 mM ferrous ion). All of the studies were done in triplicate, and the findings were expressed as mean ± standard deviation.
Using a spectrophotometer (Hewlett-Packard 8452 A Spectrophotometer) and the Folin-Ciocalteau technique, the total phenolic content of each extract was determined (SINGLETON; ROSSI, 1965). The absorbance was measured at 765 nm, and the results were reported as mg.100 g–1 gallic acid equivalent in fresh weight (GAE).

N. ABTS Method

The deactivation of the antioxidant radical cation ABTS•+, which is determined by a decrease in absorbance at 734 nm, is the basis of the ABTS technique (2,2'-azino-bis (3-ethylbenzthiazoline-6- sulphonic acid). The ABTS procedure was carried out according to (Re et alinstructions. .'s .,1999). 7 minutes after the extract was added, the absorbance was measured at 734 nm. The blueberry's total antioxidant activity was measured in moles of TEAC per kilogramme of fresh weight (Trolox-equivalent antioxidant capacity).

O. DPPH method

One of the few stable and commercially available organic nitrogen compounds is the 2.2-diphenyl-1-picrylhydrazyl (DPPH•) radical. The approach is based on the deactivation of the DPPH• radical in fruit extracts by chemicals having antioxidant capabilities, which is measured at 515 nm. (Kim et al. 2002). described how to perform the DPPH procedure At 515 nm, 30 minutes after the addition of each extract, the absorbance of 100 M DPPH• radical (2.9 mL) dissolved in 80 percent methanol was measured. The total antioxidant activity of blueberries was measured in mol.100 g–1 of TEAC and expressed in fresh weight.

P. FRAP Method

The FRAP approach is based on the direct evaluation of antioxidant (reducing) ability through the reduction of the complex Fe3+/ tripyridyltriazine (TPTZ) to Fe2+ at acidic Ph(3.6), as described by(Benzie and Strain, 1996), with improvements by (Arnous, Makris, and Kefalas ,2002). The reducing power, in fresh weight, was expressed as Mol.100 g–1 of TEAC, and the absorbance was measured at 620 nm.

The ORAC assay was employed in this investigation, which is an often used and contrasted analysis in the scientific literature (Thaipong,K et al., 2006). The assay involves oxidising fluorescein in the presence of an APPH radical.Fluorescein was diluted in 75mM phosphate buffer (pH 7.4) and stored at 20°C for no more than four weeks. The final dilution used was 6 nM. The calibration curve was made with Trolox C 0.25mM. Dilutions of APPH (127 nM) and fluorescein in phosphate buffer (75 mM) were used to prepare APPH and fluorescein (pH 7.4).The determination was carried out using a Biotek Instruments, Inc. Synergy HT multidetec microplate reader (Winooski, VT, USA). The wavelength of fluorescein was measured at 485/20 nm. The radical's absorption capacity was calculated using the same method as previously published (D´avalosA et al.,2004)with a few minor tweaks.

III. DETERMINATION OF ANTIMICROBIAL ACTIVITY

A. Agar well Diffusion Assay

The experiment was carried out on nutrient agar plates. To acquire the lawn growth of the organism, the microbial culture was streaked on nutrient agar plates with the use of a sterile cotton swab. Then, using a cork borer (6mm), a well was drilled in the centre of the plate. With the use of a sterile pipette, an adequate quantity of extract (1 ml) was subsequently added to the well. The dishes were then placed in the refrigerator for 30 minutes at 4-5 °C to allow the extract to diffuse properly. The plates were then incubated for 24-48 hours at 37° C. The zone of inhibition was measured after incubation.(Patel N *et al.*2021).

B. Antioxidant Properties of Berry Fruits

Blueberries have a high antioxidant activity and are a good source of antioxidants in the diet (Prior, 1998).Our blueberry fruit hadhigh antioxidant content. Superior to the performance of many of the cultivars studied by (Sellappan et al.,2002) used the ABTS assay on açai, cashew apple, strawberry, passion fruit and mango) and ABTS and DPPH techniques were used by (Rufino et al., 2010). Wolfe et al. 2008 tested the antioxidant activity of 25 different fruits regularly consumed in the United States and discovered that blueberries were one of the fruits with the highest antioxidant activity in a cell culture system. There was also a strong link between total phenolic content and antioxidant activity in cells, indicating that the latter might be used as an indicator of the former. These findings point to the possibility that blueberry extracts can reduce the risk of cancer by reducing oxidative processes in cells.

IV. BERRY FRUIT BIOACTIVE COMPOUNDS: BIOLOGICAL ACTIVITY AND MEDICINAL PROPERTIES

A. Oxidative Stress Suppression

Oxidative stress, which is brought on by high amounts of free radicals, can accelerate ageing and a number of degenerative disorders. Active oxygen radical scavengers including vitamin C, phenolic compounds, flavonoids, and carotenoids interact with antioxidants found in berries.

The antioxidant content of berries is four times higher than that of other fruits and vegetables.Ten times the quantity of veggies Studies have demonstrated the antioxidant qualities of strawberries.Their capacity is closely linked to the quantity of vitamin C and phenolic chemicals they contain. (Baby B.et al., 2018).In strawberries, vitamin C is the most important antioxidant, followed by anthocyanins.hydroxycinnamic acids (mostly p-coumaric acid derivatives and flavanols) are next (Tulipani, S et al, 2008).Strawberry eating boosts antioxidant capacity in the blood and lowers oxidative stress.Increases serum vitamin C levels and decreases plasma protein levels (Romandini, S et al.,2013, Henning, S.M.et al.,2008).

B. Antimicrobial Properties

Many plants produce antimicrobial secondary metabolites as part of their normal growth process and in response to pathogen infections. People with H. pylori who ate the burdock complex for eight weeks had less urea breath, for example.Inflammatory signs were seen in the test findings when compared to the placebo. Research backs up the high amount of evidence. Oxidative stress, which is brought on by high amounts of free radicals, can accelerate ageing and a number of degenerative disorders. Active oxygen radical scavengers including vitamin C, phenolic compounds, flavonoids, and carotenoids interact with antioxidants found in berries. The antioxidant content of berries is four times higher than that of other fruits and vegetables. Ten times the quantity of veggies Studies have demonstrated the antioxidant qualities of strawberries. Their capacity is closely linked to the quantity of vitamin C and phenolic chemicals they contain.

. Blackcurrant juice has antimicrobial effects

Typhimurium Anthocyanins in this strain prevent Salmonella from adhering to human cells

in epithelial colorectal cancer cells by up to 39%(Vieira, D.R.et.al.,2014, Yen, C.H., et.al.,2018).

Daily consumption of cranberry juice has been found to fight against H. pylori infections and has shown significant bacterial suppression in clinical trials(Li, Z.X.et.al,2021).Animal studies show that berry components such as epicatechin, chlorogenic acid and quercetin effectively counteract nonsteroidal anti-inflammatory drugs mediated damage and H. pylori infection. The effect of berries on H. pylori infection is also approved in human trials by using cranberries, which contains active fractions of quercetin and epicatechin(Govers, C et.al., 2018).

Blueberries reduce the frequency of Clostridium perfringens, Enterococcus, and E. coli in the cecum, all of which are related with irritable bowel syndrome. The amount of Lactobacilli and Bifidobacteria in rats fed 4 mL/kg blueberry extract daily for six days exhibited a significant increase, confirming the prebiotic potential of blueberries(Paturi, G.et.al.,2012, Molan, A.L.et.al.,2009).

C. Neuroprotection

In a study with rats, (Duffy et al., 2008) confirmed that supplementing the diet with 2% blueberry extract for 8 weeks protected them from neurodegeneration and cognitive deficits caused by excitotoxicity and oxidative stress. This study found evidence that supplementing with blueberry extract can help prevent or treat Alzheimer's disease, as well as possibly other neurodegenerative disorders. It was also discovered that the extract can help slow down degenerative processes induced by oxidative or inflammatory processes.

A key factor is oxidative stress, which causes damage to brain macromolecules.

In neurodegenerative illnesses, this is a process. Alzheimer's disease is a widespread neurodegenerative illness that affects up to 18 million individuals globally. Polyphenols have a significant antioxidative capacity, hence they may protect against neurological illnesses if consumed(Letenneur L, et al.,2007)When compared to those who drank less or did not drink at all, those who drank three to four glasses of wine per day had an 80% lower incidence of dementia and Alzheimer's disease(Scarmeas N, et al.,2007).

Resveratrol, which is prevalent in wine, scavenges O2 - and OH• free radicals in vitro, as well as lipid hydroperoxyl free radicals; this potent antioxidant activity is likely to be implicated in the protective effect of moderate red wine consumption against dementia in the elderly. In a model of Alzheimer's disease, resveratrol suppresses nuclear factor B signalling and so protects against microglia-dependent -amyloid toxicity, and this activity is linked to SIRT-1 activation(MarkusMA,et al.,2008). It was discovered that drinking high-polyphenol fruit and vegetable juices at least three times a week may help to prevent the beginning of Alzheimer's disease(Dai Q,et al.,2006) Polyphenols found in fruits and vegetables appear to be valuable potential neuroprotective agents due to their capacity to inhibit oxidative stress(Singh M,et al .,2008).

D. Anticancer Properties

The antioxidant impact of berries coincides with its anticancer potential, according to studies in vitro and in vivo (Castro, D.et.al.,2015 ,Skrovankova, S.et.al 2015).Scavenging reactive oxygen species is part of the antioxidant process.ROS are reactive oxygen species (ROS) that cause oxidative damage to biological macromolecules like DNA and RNA. DNA and RNA are two types of genetic material. The accumulation of oxidative DNA damage aids in the genesis of cancer. oxidative stress is one of the major causes of tumours, and as a result, oxidative stress is one of the major causes of cancer.

Increasing the rate of carcinogenesis (Sosa, V.et.al,2013). Strawberry extract has been shown to drastically reduce tumour growth. volume and lengthen the longevity of the mouse model (Somasagara, R.R.et.al,2012). Strawberry bioactive chemicals in a mouse model of azoxymethane/dextran sodium sulfate-induced colon carcinogenesis (Shi, N.et.al,2012)model Strawberry also has an anti-proliferative impact, according to an in vitro study.

On human colon, prostate, and oral cell lines, extracts with ellagic acid (Zhang, Y.et.al,2008). An ingestion weighing 60 gper day of freeze-dried strawberry powder reduced histological grade levels of several pro-inflammatory proteins in premalignant lesions in more than Esophageal dysplasia affects 80 percent of patients (Chen, T.et al,2012).

Anthocyanins from blackberries inhibited cancer cell growth by modifying cellular signalling pathways, such as modulating the expression of activating protein-1 (AP-1) and nuclear factor-kB (NF-kB), two essential proteins that coordinate cell proliferation, vascular endothelial cell proliferation, and vascular endothelial cell proliferation.

COX-2, and growth factor (Duthie, S.J,et al.2007). Furthermore, blackberry quercetin is a powerful antioxidant.Experimental animal models and human cancer cell lines revealed anti-carcinogenic capabilities (HT29 and Caco-2) (Agullo, G.et.al,2007).

(Seeram et al. 2006) studied the ability of blueberry extracts to inhibit tumour cell proliferation in the oral cavity, breast, colon, and prostate, concluding that the activity was dose-dependent and different among cell types. Furthermore, the extracts induced apoptosis in colon cancer cell cultures.

The effect of blueberry juice on cell death and cell cycle interruption in human cancer cells of the stomach, breast, prostate, and intestine was studied by (Boivin et al.2007). These researchers discovered that juice, particularly juice from cultivars of the "lowbush" group, had a strong capacity for suppressing cell proliferation. More than caspase-dependent apoptosis, the mechanism of action appears to be connected to cell cycle disruption.

Polyphenols affect pro-carcinogen metabolism through regulating the expression of cytochrome P450 enzymes involved in their conversion to carcinogens. They may also make it easier for them to be excreted by upping the expression of phase II conjugating enzymes. The toxicity of polyphenols may be to blame for the stimulation of phase II enzymes (ScalbertA,atal,2005). In the body, polyphenols can create potentially hazardous quinones, which are also substrates for these enzymes. Polyphenol consumption could then activate these enzymes for self-detoxication, resulting in a general improvement in our defences against hazardous xenobiotics. (Talalay P, et al 1988).Teacatechins in the form of capsules have been shown to have cancer-preventive effect in males with high-grade prostatic intraepithelial neoplasia (PIN) by blocking the conversion of high-grade PIN to cancer (Khan N et al, 2005).

E. Blood Pressure

Polyphenols and flavonoids have been shown to be helpful in the treatment of cardiovascular illnesses, such as hypertension. Anthocyanins and anthocyanin-rich berry eating have been linked to a considerable reduction in blood pressure in some studies(Clark, J.L.et al., 2015, Vendrame, S.et al., 2019). After 12 days of ingestion of a blackcurrant extract containing either 105, 210, or 315 mg/day of anthocyanins, a group of 15 athletes with the two higher anthocyanin doses showed a substantial decrease in arterial pressure (Cook, M.D.et al., 2017).During an eight-week study, 45 prehypertensive participants were given black raspberry powder, and the group receiving 2.5 g raspberry powder per day saw a significant decrease in systolic blood pressure (SBP) (Jeong, H.S.et al.,2016). Zhu and colleagues (Zhu, Y.et al., 2017)conducted a meta-analysis of various randomised clinical studies and found no significant effect of blueberry eating on either systolic or diastolic blood pressure. For six weeks, older adults were given either whole wild blueberry powder (1 to 2 g per day) or 200 mg of blueberry extract (2.7, 5.4, or 14 mg anthocyanin content). The extract, which provided a larger dose of anthocyanins than whole berry powder, resulted in a significant reduction in SBP (Whyte, A.R.et al., 2018).

F. Diabetes

With the beginning of hyperglycemia and, eventually, diabetes, impaired glucose metabolism causes physiological imbalance. Diabetes is divided into two types: type 1 and type 2. Several physiological indicators have been found to be related in studies.

In diabetic situations, the body's chemistry changes(Rizvi SI et al.,2001,2005) In the long run

Diabetes has a number of side effects, including the progression of certain diseases.

Complements like retinopathy, which affects the eyes and can lead to blindness.

Nephropathy, a condition in which the kidneys' functions are disrupted

or agitated, as well as neuropathy, which is linked to an increased risk of amputations, foot ulcers, and other symptoms of autonomic dysfunction, sexual dysfunctions are included. The anti-diabetic effect has been documented in a number of researches.

The anti-diabetic properties of tea catechins have been studied(Rizvi SI et al ,2005, Rizvi S I et al,2001) Polyphenols may influence glycemia through a variety of methods, including inhibiting glucose absorption in the gut or peripheral tissue uptake. At a 10 mg/kg food intake, diacetylated anthocyanins had hypoglycemic effects with maltose as a glucose source, but not with sucrose or glucose(Matsui T, et al ,2002).This shows that the inhibition of -glucosidase in the gastrointestinal mucosa is the cause of these effects. Catechin was found to inhibit -amylase and sucrase in rats at doses of about 50 mg/kg diet or higher.

G. Obesity

Obesity is difficult to cure and is a leading cause of diabetes and cardiovascular disease. Natural plant extracts have been proposed as an option for long-term weight control (178 Song, Y.et al.,2013), because pharmacological therapy of obesity causes many adverse effects and has little long-term efficacy. C57BL/6J mice fed a high-fat diet with anthocyanins were found to be less obese than mice fed a high-fat diet without anthocyanins (Tsuda, T et al., 2003). Weight gain and fat accumulation in mice fed a high-fat diet were not affected by blueberry juice or freeze-dried blueberries. Anthocyanin extracts from blueberries, on the other hand, lowered body weight and fat formation considerably (Prior, R.L.et al., 2010).The anthocyanins in blueberries promoted the transcription of the peroxisome proliferator-activated receptor (PPAR, which participates in energy homeostasis regulation) (Seymour, E.M et al., 2010), which is linked to improved insulin resistance, fat stimulation metabolism, and fat storage inhibition.

After a three-week ingestion of strawberry powder, in obeservedparticipants lipid profile and inflammatory indicators improved in a human investigation (Zunino, S.J.et al., 2012). Another study found that eating freeze-dried strawberries for 12 weeks reduced inflammatory markers in obese adults with osteoarthritis, as well as tumour nuclear factor- and lipid peroxidation products (Basu, A.et al,2018). Strawberry eating reduced cardiovascular disease and diabetes risk factors in obese volunteers, suggesting that strawberries could be used as a therapeutic food to combat obesity-related disease.

H. Cardiovascular Disease

Blueberry phenolic compounds revealed the role in the decrease of one of the risk factors for cardiovascular disease. Supplementing swine with blueberry extract (Vacciniumcorymbosum cv. Jersey) lowered total cholesterol, LDL, and HDL levels, according to a study. The biggest reduction was shown with blueberry at a concentration of 2%, with total cholesterol, LDL, and HDL lowered by 11.7 percent, 15.1 percent, and 8.3 percent, respectively(Kalt, W et al.,1997).

Polyphenol consumption has been shown in a number of studies to reduce the risk of coronary heart disease(Renaud, S et al., 1992, Dubick, MA et al., 2001, NardiniM,et al., 2001).Atherosclerosis is a long-term inflammatory condition that affects the arteries. in the prone-to-lesion areas of medium-sized arteries Atherosclerotic For decades, lesions may be present yet clinically quiet. Activating and causing pathological disorders, such as acute myocardial infarction, unstable angina, or sudden cardiac death are all symptoms of acute myocardial infarction. Death(Vita JA,et al.,2005). Polyphenols are powerful inhibitors of LDL oxidation, which is thought to be a crucial factor in heart disease.

There are more methods that Polyphenols have been linked to a reduction in the risk of cardiovascular disease. Antioxidant, anti-platelet, and anti-inflammatory properties as well as enhancing endothelial function and raising HDL Polyphenols may also help to maintain atheroma stability.(García-Lafuente A et al.,2009).

V. CONCLUSION

Berry fruits are well recognized as natural functional goods due to their diverse composition of bioactive chemicals and their health-promoting characteristics, which are mostly due to their antioxidant activity.One of the most significant families of phytochemicals, phenolic compounds has both functional and health-promoting effects. Fruits are excellent sources of these compounds, and improved methodology for extracting, isolating, separating, identifying, and quantifying the full range of phenolic content in fruits is critical for understanding the health potential of different fruits as well as good sources of these compounds.

The properties of distinct berry fruit species show significant variances in the types of bioactive chemicals they contain. Such changes can be seen in the content as well as the qualitative composition of those molecules. Phenolic chemicals and vitamin C are credited with the greatest health advantages.

Berries have significant health advantages in both in vitro and in vivo investigations. They include powerful antioxidants that protect against inflammatory disorders, metabolic disorders, cardiovascular diseases, and can even lower the risk of cancer. They have antibacterial and neuroprotective effects as well. The interaction of berry phenolics with the microbiota is important for berry phenolic bioavailability and contributes to gut health.Berries have the potential to be used as pharmaceutical agents to treat a variety of diseases. Future clinical trials will be needed to investigate and improve the bioavailability of phenolic compounds found in berries, as well as to add to the evidence that berries active chemicals can be used as medicinal foods to treat a variety of ailments.

The medications derived from these fruits, in particular, are being studied in the fight against cancer, which is today's most prevalent disease. People also eat them due of their distinctive colours, forms, and flavours (as in the food industry, pastry and cake, juice, liquor, tea, etc.). The number of berry fruit-related garden installations has recently expanded. These fruits are frequently shown on television and in ads. As a result, people are more influenced to berries.

However, no adverse or toxic effects (i.e., chemical, haematological, or urinary effect) have been linked to the consumption of berries, berry juices, or other extracts, particularly aronia berries and aronia products, in vivo or in vitro (Kulling and Rawel, 2008), suggesting that the phenolic antioxidants found in berries are natural gifts for human health. However, the phenolic component content of berries and berry products is not always fully defined, and more research is needed to determine the therapeutic levels of various berry products for future clinical trials.

Furthermore, more research is needed to fully comprehend the favourable benefits described thus far from a mechanistic standpoint. As a result, more emphasis should be placed on the establishment of well-controlled and high-quality clinical trials.

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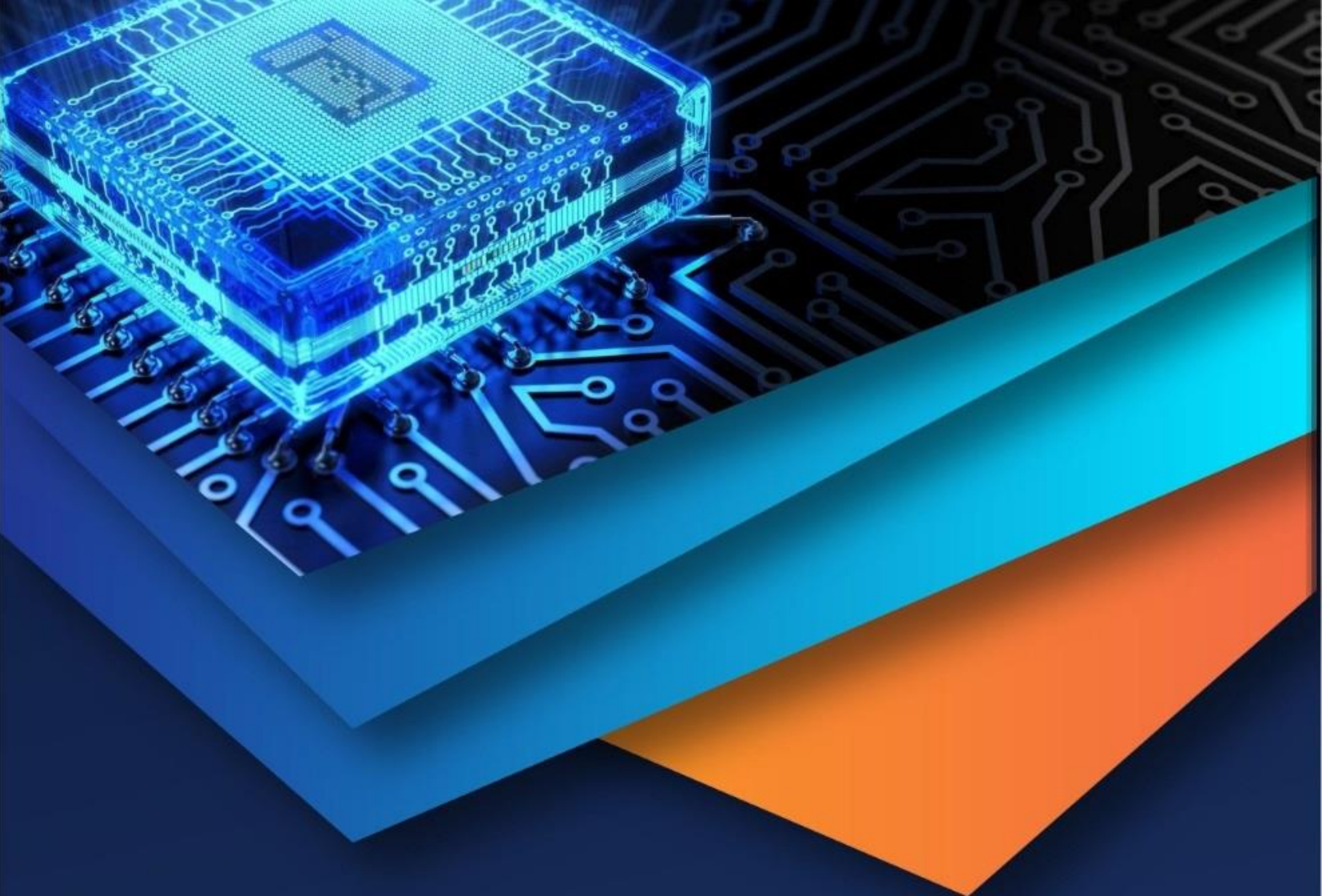

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