Polyphenols Target Apoptosis and Cell Cycle Regulation in Various Cancer Models: Emphasis on Flavonoid Subclasses

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Cancer remains a major global health concern, driving the ongoing search for safer and more effective therapeutic agents. Naturally occurring polyphenolic compounds, including flavonoids, have gained considerable attention for their diverse pharmacological activities, particularly their ability to modulate cancer cell proliferation and apoptosis. This review summarizes current evidence on the anticancer potential of polyphenols and their subclasses across various human cancer cell lines, emphasizing their mechanisms in inducing programmed cell death and regulating cell cycle progression. Key flavonoids such as apigenin, quercetin, and kaempferol, along with other polyphenols like resveratrol and curcumin, exhibit significant anticancer effects through the activation of intrinsic and extrinsic apoptotic pathways and modulation of key cell cycle checkpoints. In vitro studies consistently demonstrate dose-dependent cytotoxicity, mitochondrial membrane disruption, caspase activation, and regulation of p53, Bcl-2, cyclins, and CDKs in breast, colon, lung, prostate, and cervical cancer models. Despite encouraging preclinical outcomes, limitations such as poor bioavailability, inter-model variability, and lack of standardized protocols persist. Furthermore, synergistic interactions with conventional chemotherapeutics and advancements in delivery systems and molecular profiling tools may enhance their clinical relevance. Overall, polyphenols, particularly flavonoids, represent a promising class of anticancer agents warranting further translational and clinical exploration.

Keywords: Anticancer Therapy; Apoptosis; Cancer Cell Lines; Cell Cycle Arrest; Flavonoids; Natural Compounds; Polyphenols.

Cancer is a worldwide health issue that transcends national borders. In addition to environmental and medical causes, it occurs as a result of widespread DNA damage caused by ionising and UV radiation. Overall, the most often diagnosed cancers are stomach (7.81%), colorectal (9.7%), lung (12.7%), and breast (10.9%). Over the next 20 years, there will likely be a 50% increase in new cancer cases worldwide.[1] Numerous obstacles confront global cancer care, particularly in low- and middle-income (LMIC) nations. Access to quality, affordable cancer care including screening, skilled staff, standard treatments, and supportive services is often limited in many regions.[2] In 2020, over

19 million people were diagnosed with cancer, resulting in nearly 10 million deaths globally.[3]By 2040, an estimated 28 million additional cases and 16 million fatalities are predicted.[4,5]In 2019, the cost of cancer treatment alone was estimated to be US$1.2 trillion per year, or about 2% of the world's total domestic product.[6]Although they can be somewhat effective, traditional cancer treatments like chemotherapy and radiation therapy are frequently linked to serious side effects, poor specificity, and the development of resistance. The pressing need for safer and more efficient therapeutic alternatives is highlighted by this increasing burden. Natural products' structural variety, bioactivity, and comparatively low toxicity have made them valuable sources of new anticancer medicines in recent years. Because they are widely accessible, efficient, and possess anti-cancer qualities, natural compounds are the most effective against cancer. Natural components have been a defining feature of the history of anticancer medication development. Numerous widely used anticancer drugs are derived from natural sources, such as paclitaxel from plants, irinotecan, vincristine, etoposide, marine-derived bleomycin, and actinomycin D and mitomycin C from bacteria. For the foreseeable future, some of these substances will remain essential components of cancer treatment.[7]Flavonoids and polyphenols secondary metabolites found in fruits, vegetables, and medicinal plants show strong potential in cancer prevention and treatment, including compounds like catechin, quercetin, ellagic acid, and stilbenes. *Phyllanthus emblica, Flemingia vestita, Apium graveolens, Petroselinum crispum,* and other medicinal plants are among the many plant sources from which natural flavonoids are extracted. Polyphenols are a broad category of compounds that include lignans, stilbenes, phenolic acids, and flavonoids. Flavonoids, a key subclass of polyphenols, are classified into flavonols, flavones, isoflavones, flavanones, anthocyanidins, and chalcones based on structure.[8]They exhibit anti-inflammatory, antioxidant, and anticancer properties by inducing apoptosis in breast, colorectal, and prostate cancers, inhibiting nucleoside diphosphate kinase-B in lung, bladder, and colon cancers, and blocking the NF-kB pathway to halt cell proliferation and induce cell cycle arrest.[9]These compounds modulate key

signaling pathways linked to tumor progression, particularly apoptosis and cell cycle regulation, which are pivotal targets in cancer therapy screening.[10]This review evaluates flavonoids and polyphenols studied in cancer cell lines, emphasizing their role in inducing apoptosis and cell cycle arrest, while highlighting their potential in anticancer drug development.

MATERIALS AND METHODS

A comprehensive and systematic literature search was conducted using major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar, focusing primarily on original research articles, review papers, and meta-analyses published using keywords such as *"flavonoids," "polyphenols," "apoptosis," "cell cycle arrest," "cancer," "molecular targets,"* and specific compound names like *quercetin, genistein, curcumin,* and *resveratrol.* Only English-language publications were considered. To give a comprehensive picture of the molecular functions and therapeutic implications of these phytochemicals, included were studies that combined clinical research with *in vitro* and *in vivo* cancer models. Emphasis was placed on research identifying molecular targets, dose-dependent responses, synergistic effects, and selectivity toward cancer cells. Articles that lacked mechanistic insights or reproducible data were excluded. Information was extracted and synthesized based on compound type, cancer model, apoptotic/cell cycle effects, and therapeutic potential. References were managed and organized using Mendeley, and PRISMA guidelines were loosely followed to ensure the rigor and transparency of the review process.

Classification and Sources

Approximately 8000 polyphenols have been identified, categorized into flavonoids (flavones, flavonols, isoflavones, flavanones, anthocyanins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes (resveratrol, piceatannol), lignans (sesamol, pinoresinol, sinol, enterodiol), and tannins (hydrolysable, non-hydrolyzable, and condensed) (The structural classification of flavonoids is depicted in Fig.1).[11-13]Dietary polyphenols mainly include flavones, isoflavones, catechins, and phenolic

acids.[11]Phenolic acids, with aromatic rings and carboxylic acid groups, are abundant in plants and known for their antioxidant properties that help prevent cardiovascular diseases.[11,14,15]Their aromatic rings act as reducing agents by donating electrons and neutralizing free radicals.

In plant-based diets, the primary sources of phenolic acid are leafy vegetables, fruit skins, and seeds. There are several applications for these phenolic acids in cosmetics, medicine, and food.[16]These compounds vary in structure from simple molecules to complex polymers, yet all possess hydroxylated aromatic rings, which contribute to their antioxidant and bioactive properties. Understanding the structural diversity of flavonoids and polyphenols is essential, as their anticancer mechanisms including apoptosis induction and cell cycle arrest are often closely linked to their chemical nature and cellular interactions. Flavonoids and polyphenols are widely distributed in various dietary and medicinal plant sources, making them readily accessible through everyday nutrition and traditional herbal remedies (Table 1).

Flavonoids

Flavonoids, a major class of polyphenolic plant metabolites, possess a 15-carbon skeleton with two aromatic rings linked by a three-carbon chain.[17]Based on oxidation, conformation, and B-ring attachment, they are classified into flavones (chrysin, apigenin, baicalein), flavonols (quercetin, kaempferol), isoflavones (daidzein, glycitein), flavan-3-ols (gallocatechin, catechin, epicatechin), flavanones (hesperetin, naringenin), and anthocyanidins (delphinidin, peonidin, cyanidin, pelargonidin).[11]Found in berries, onions, tea, grapes, apples, and cocoa, flavonoids offer cell-signaling, anti-thrombogenic, and neuroprotective effects.[18]

Flavonols

A subclass of the flavonoid family, flavonols are made up of carbonyl C4 and double bonds between C2 and C3. Plant flavonols like myricetin, kaempferol, and quercetin are commonly found in fruits and vegetables, including tomatoes, lettuce, onions, and kale.[19]Clinical trials have demonstrated that these flavonols may be used to prevent and treat heart disease, blood coating, cell regeneration, human gingival disease, and other heart-related conditions.[12]

Isoflavones

One class of phytoestrogens that are nonsteroidal substances obtained from plants are isoflavones.[20]The Fabaceae family is the source of isoflavones. In place of the phenyl group, isoflavones have a structure consisting of two phenyl-4H-1benzopyr-4-one.[12]Isoflavones are produced by the phenylpropanoid pathway, which helps higher plants produce flavone families. Isoflavones can be found in soybeans and their byproducts. Soybeans have a high isoflavone concentration because they include two important components, genistein and daidzein.[21]Isoflavones are believed to have chemo-protective qualities and may be used to treat menopause-related symptoms, according to research.[11]According to studies, it also help treat osteoporosis, heart-related conditions, cancer (prostate and breast), and a number of hormonal diseases.

Flavanones

Flavanones, which make up a minor portion of flavonoids, are found in citrus fruits, tomatoes, and certain aromatic plants. In addition to its flavouring properties, they are thought to have a significant role in human health. Flavanones include naringenin from grapefruit, hesperidin from oranges, and eriodyctiol from lemons.[22]In addition, flavanones go through a number of chemical reactions, including hydroxylation, glycosylation, and o-methylation.[12]Flavonoids have been shown to be abundant in bergamot juice, also known as citrus bergamia.[23]This indicates that bergamot extract lowers serum cholesterol, offering cardiovascular benefits by improving lipid profiles, lipoproteins, and reducing atherosclerosis risk. Prolonged use over six months has shown cholesterol-lowering effects, with LDL monitoring aiding in detecting cardiovascular events.[14]

Anthocyanidins

The primary pigments that give fruits and vegetables their red, pink, and purple colours are called anthocyanidins.[24]Anthocyanidins present in the epidermis give fruits, vegetables, and flowers their color. They are abundant in berries, cherries, beets, radish, and strawberries,[25] with aronia berries being most consumed for their strong antioxidant activity.[26]Chocolate berries contain flavanols, kaempferol, cyanidin glycosides, and other beneficial compounds.[12]Over time, berries, honey,

and olive oil have all demonstrated significant health benefits.

Flavones

Broccoli, celery, parsley, and grains are common sources of flavones. The outer coating of citrus fruits also contains significant amounts of them. Flavones were found to have an inverse effect on CAD.[27]One of the flavones, tangerine, inhibits platelet synthesis, which encourages the body's smooth muscles to multiply, resulting in atherogenesis and restenosis. Consuming citrus fruits that contain nobiletin can help reduce inflammation and cancer. Numerous studies have demonstrated that nobiletin promotes platelet activation. It also inhibits the phosphorylation of MAPKs and protein kinases.[28]

Other polyphenols

Polyphenols like tannins, lignans (e.g., sesamol, enterodiol), and stilbenes (e.g.,

resveratrol) have diverse medicinal and commercial applications based on their modes of action (Fig.1).[11]

Stilbenes

Stilbenes (C $_{,,}$ H $_{,}$), phenol-derived metabolites, are widely studied for their biological activity and health benefits.[29]Stilbenes are compact chemical compounds containing one phenyl group and a core ethylene portion. At the extremities of the carbon double bonds lies the phenyl group.[30]Tran's stilbenes is the colloquial term for stilbenes. Auguste Laurent, a French scientist, made the discovery of stilbenes in 1843. Their overall health advantages are currently being investigated, as are their bioavailability, metabolic rates, and absorption in the body.[29]

Lignin

Numerous plant tissues include the complicated chemical compound family known
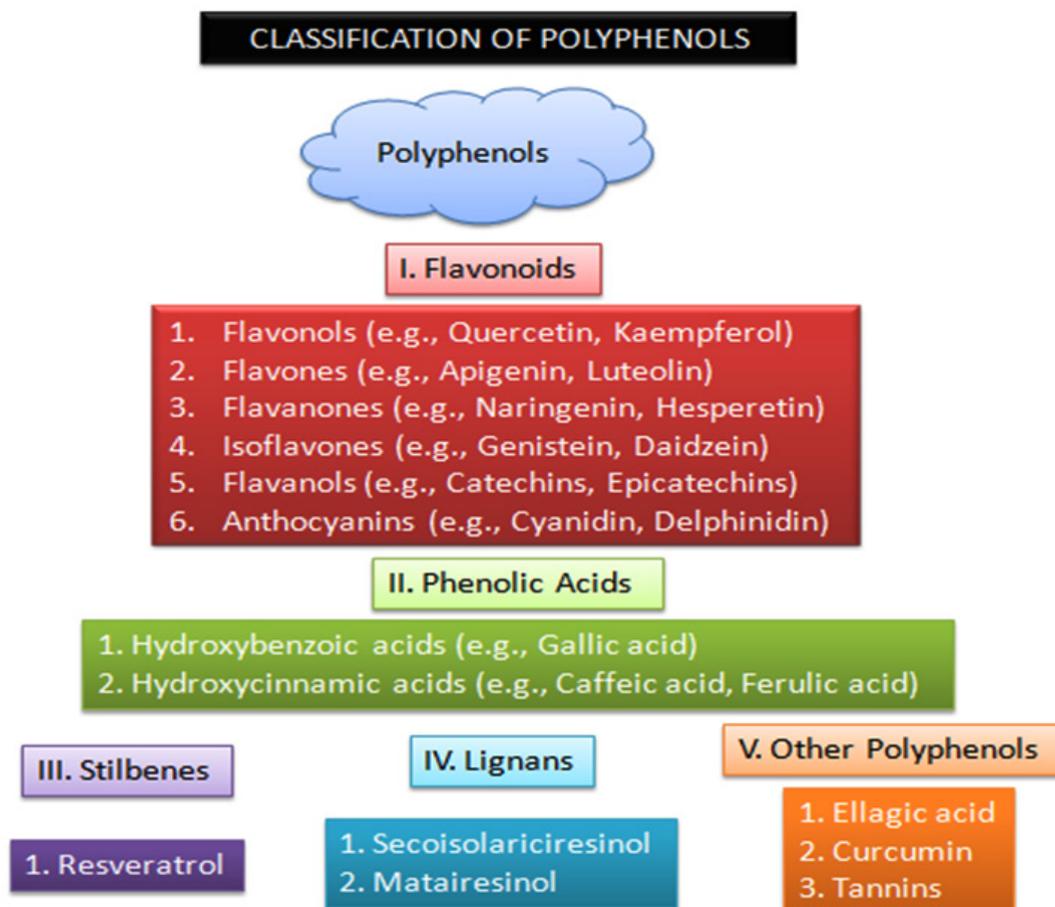
Fig.1. Classification of Polyphenols

as lignin. Because it encourages the formation of the cell wall, lignin is especially important in plants and trees.[31] They are crucial because they give trees stiffness and strength. A P Candolle, a Swiss botanist, made the initial identification of lignin in 1813. A few numbers of signal precursors are used to create the heterogeneous polymers known as lignin, which then go through various cross-linking processes.[12] Coniferyl alcohol, sinapyl alcohol, and paracoumaryl alcohol are the three forms of cross-links that are generated from phenylpropane.[31] Foods high in lignin include apples, peaches, tomatoes, flaxseeds, and some berries.

Bioavailability and metabolism considerations

Despite their promising anticancer properties, the bioavailability and metabolism of flavonoids and polyphenols present significant challenges for their clinical application. These compounds often exhibit poor absorption, rapid metabolism, and limited systemic availability when consumed orally.[32] Among the factors influencing their absorption in the gastrointestinal tract include molecular size, glycosylation, solubility, and interactions with gut flora. After absorption, flavonoids and polyphenols undergo extensive phase I and II metabolism in the intestinal wall and liver, potentially reducing their biological activity. This metabolism includes oxidation, methylation, sulfation, and glucuronidation.[33] Furthermore, the effects of conjugated metabolites, which are produced during metabolism, may differ or be less pronounced than those of the parent substances. Because it has the ability to either activate or degrade polyphenols into bioactive or inactive metabolites, the gut microbiota plays a crucial role.[34] Furthermore, many flavonoids and polyphenols have a short plasma half-life, requiring high or repeated dosing to maintain therapeutic levels, which is often impractical. Many approaches, such as the utilisation of phospholipid complexes, liposomes, nanoformulations, and co-administration with bioenhancers like piperine, have been investigated to get around these restrictions. Enhancing solubility, stability, absorption, and targeted delivery are the goals of these strategies.[35] Maximising these medications' therapeutic potential and ensuring a smooth transition from *in vitro* research to *in vivo* models

and clinical settings requires an understanding of their pharmacokinetic behaviour.

Mechanisms of Cancer Cell Cycle Regulation and Apoptosis

Overview of apoptosis pathways

Apoptosis is a regulated mechanism essential for eliminating damaged or malignant cells and maintaining tissue homeostasis.[36] It proceeds via intrinsic and extrinsic pathways. The intrinsic pathway is triggered by internal stress like DNA damage or oxidative stress, leading to mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome c, which forms the apoptosome with Apaf-1 and pro-caspase-9, activating caspase-3. The Bcl-2 family regulates this balance. The extrinsic pathway is initiated by death ligands (e.g., FasL, TNF-á, TRAIL) binding to death receptors (Fas, TNFR1, DR4/DR5), forming the DISC complex that activates caspase-8 or -10. The apoptotic process is then triggered by these initiator caspases activating effector caspases, including caspase-3. Through mitochondrial participation, the apoptotic signal may be increased by the interaction of the intrinsic and extrinsic pathways. Determining how flavonoids and polyphenols can specifically cause apoptosis in cancer cells requires an understanding of these pathways.[37]

Role of key regulators: Bcl-2, Bax, caspases, cytochrome c

Key regulators of apoptosis include Bcl-2, Bax, caspases, and cytochrome c. In the intrinsic pathway, the balance between pro- and anti-apoptotic Bcl-2 family proteins is crucial. Bcl-2 maintains mitochondrial membrane integrity by inhibiting apoptogen release, while Bax promotes MOMP, leading to cytochrome c release into the cytosol. The apoptosome is formed when cytochrome c enters the cytoplasm and binds to pro-caspase-9 and Apaf-1 to activate initiator caspase-9. The breakdown of cellular components is then regulated by effector caspases, primarily caspase-3, which cleave structural and repair proteins, finally resulting in apoptosis. Caspases are cysteine proteases that are produced as dormant zymogens and undergo cascading activation. Cancer cells frequently exhibit altered Bcl-2 and Bax functional status and relative expression levels, which contribute to apoptotic resistance.

Therefore, a variety of anticancer drugs, including flavonoids and polyphenols, work by altering these regulators, which tilt the scales in favour of apoptosis by downregulating Bcl-2, upregulating Bax, and activating caspases. Clarifying how phytochemicals can specifically cause cancer cells to die without harming healthy tissues requires an understanding of the functions of these compounds.[38]

Cell cycle phases and checkpoints

The highly regulated cell cycle allows for precise cell division and replication. The cell cycle consists of G , S, G, , and M phases. G involves cell growth and preparation for DNA replication, while the S phase focuses on DNA synthesis. The G, phase, which gets the cell ready for mitosis, and the M phase, which is when cell division happens, come next. A number of cell cycle checkpoints, which keep an eye on cellular circumstances and guarantee the integrity of the genome, strictly regulate the movement through these stages. The G /S checkpoint detects DNA damage before replication, while the G, /M checkpoint ensures DNA is fully and accurately replicated before mitosis. Moreover, proper chromosomal alignment and attachment to spindle fibres are maintained throughout mitosis by the spindle assembly checkpoint (SAC). Cyclins, CDKs, and their inhibitors regulate these checkpoints. For example, the tumor suppressor p53 halts the cell cycle upon DNA damage by inducing p21, a CDK inhibitor that blocks cyclin-CDK complexes. In cancer, these checkpoints are often dysregulated, allowing uncontrolled proliferation. Natural compounds like flavonoids and polyphenols have been shown to interfere with important phases of the cell cycle, particularly by causing arrest at the G€ /G or G, /M phases, which inhibits cancer growth and encourages apoptosis.[39]

Key regulators - p53, p21, CDKs, cyclins

Cell cycle progression is controlled by a network of important regulatory proteins, including as cyclins, p53, p21, and cyclin-dependent kinases (CDKs). These proteins work together to preserve proper cell division and genomic integrity. The tumor suppressor p53, known as the "guardian of the genome," maintains cellular homeostasis by activating genes like p21 in response to DNA damage or stress. The potent CDK inhibitor p21 binds to cyclin-CDK complexes and prevents them from functioning, particularly those crucial for the G /S and G, /M transitions. By essentially stopping the cell cycle, this inhibition enables the cell to either repair damaged DNA or, in the event that repair is not possible, to undergo apoptosis. Cyclins bind to CDKs at specific cell cycle phases to activate them: cyclin D-CDK4/6 drives G progression, cyclin E-CDK2 facilitates the G /S transition, and cyclin A-CDK2 promotes S-phase progression. In a similar manner, cyclin B-CDK1 regulates the M phase of mitosis. A common feature of many malignancies is the dysregulation of cyclins or the overactivation of CDKs, which results in unchecked cell division. Flavonoids and polyphenols have been shown to modulate the expression or activity of these key regulators upregulating p53 and p21, and downregulating cyclins and CDKs thereby inducing cell cycle arrest and exerting anticancer effects.[40]

Table 1. Dietary and Medicinal Plant Sources of Flavonoids and Polyphenols

Source Type	Examples	Key Compounds
Fruits	Apples, berries, grapes, oranges, pomegranates	Quercetin, anthocyanins, catechins
Vegetables	Onions, kale, spinach, broccoli	Flavonols, flavones
Beverages	Green tea, black tea, red wine, coffee	EGCG, resveratrol, chlorogenic acid
Whole Grains	Oats, barley, brown rice	Phenolic acids, lignans
Nuts & Seeds	Flaxseeds, walnuts, sesame seeds	Lignans, tannins
Medicinal Plants	*Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Ginkgo biloba, Scutellaria baicalensis, Glycine max* (soy)	Curcumin, EGCG, baicalin, genistein, daidzein
Herbal Remedies	Plant extracts, powders, capsules	Mixed polyphenols and flavonoid-rich fractions

Screening Methods in Cancer Cell Lines

In vitro studies on anticancer agents, including flavonoids and polyphenols, commonly employ a range of established human cancer cell lines (Table 2) that represent different tissue origins and tumor types. These cell lines serve as reliable models for understanding the cellular and molecular mechanisms underlying cancer progression and treatment responses

In vitro assays for apoptosis and cell cycle analysis

A variety of *in vitro* assays are employed to evaluate the apoptotic and cell cycle-modulating effects of flavonoids and polyphenols in cancer cell lines (Table.3).

RESULTS

Cancer models - Flavonoids cause cell cycle arrest and death

Flavonoids are well documented for their ability to alter important signaling pathways that contribute to cancer growth, specifically by inducing cell cycle arrest and death. Their structural subclasses, each with distinct bioactivities in cancer cell lines, have a considerable influence on these effects.

Flavones

In a variety of models, flavones including luteolin and apigenin have shown strong anticancer effects. Apigenin induces apoptosis in breast and prostate cancer by activating the intrinsic pathway, inhibiting Bcl-2, and upregulating Bax and caspase-3. Additionally, it inhibits cyclin B1 and CDK1, which causes cell cycle arrest at the G, /M phase. By inhibiting PI3K/Akt signaling and triggering caspases, lutein has comparable effects on lung and colon cancer cells. G phase arrest results from changes to cyclin D1 and p21. The effects of luteolin, a dietary flavonoid, on HT 29 human colon cancer cells, with an emphasis on cell cycle regulation and death. In a dose-dependent fashion, they reduced the number of viable cells and inhibited DNA synthesis. G phase arrest was caused by the rapid suppression of CDK4 and CDK2 activity by about 38% at 40 ìM in 2 hours. This was accompanied by a drop in cyclin D levels and phosphorylation of the retinoblastoma (Rb) protein. The 24-hour therapy substantially accelerated G, /M arrest by suppressing cyclin

B and CDC2 activity. Luteolin enhanced PARP cleavage and activated caspases 3, 7, and 9, while downregulating anti-apoptotic proteins such as p21^CIP1/WAF1, survivin, Mcl-1, Bcl-x_L, and Mdm2, promoting p53-independent apoptosis and cell cycle arrest.[59] In SW480 and Caco-2 colon cancer cells, seven apigenin analogues acacetin, chrysin, kaempferol, luteolin, myricetin, naringenin, and quercetin were found to induce G, /M cell-cycle arrest. They employed DNA flow cytometry to determine that quercetin, kampherol, and luteolin likewise caused a significant G, /M arrest at 5–30/ μM, In contrast, acacetin and chrysin boosted the G, /M population in SW480 cells in a dose-dependent manner (0–80/ μM over 48 hours). The impact diminished when the concentration exceeded 40 μM. Conversely, the Caco 2 line did not exhibit notable arrest under similar conditions . With the exception of myricetin and naringenin, the majority of analogues significantly reduced cell numbers over time and at varying doses. Furthermore, when these analogs (5–30/ μM) were combined with 20/ μM apigenin, there was an additive effect a further ~22/ % increase in G, /M arrest compared to apigenin alone, though this synergy became antagonistic at higher concentrations (>/ 40/ μM) High doses of individual analogs or their combinations also decreased cell viability, as measured by Trypan blue exclusion. Interestingly, cellular uptake of some analogs increased at e"40/ μM, but this did not correlate with their cell-cycle effects. Collectively, these findings suggest that apigenin-related flavonoids can cooperatively induce G, /M arrest and reduce proliferation in colorectal cancer cells, highlighting their potential for combinatorial chemoprevention strategies.[60]

Flavonols

Flavonols like quercetin and kaempferol are well-studied for their anticancer effects, notably inducing cell cycle arrest and apoptosis. Quercetin significantly inhibits MCF-7 breast cancer cell growth by triggering apoptosis and synchronized cell cycle arrest. Treatment with quercetin (IC… € H"/ 92/ μM at 48/ h) initially induced a transient M phase accumulation, evidenced by increased mitotic index and heightened cyclin/ B1/Cdc2 kinase activity, followed by sustained G, arrest characterized by reduced cyclin/ B1 levels and diminished Cdc2 activity. Simultaneously,

Table 2. Commonly Used Human Cancer Cell Lines in Screening Flavonoids and Polyphenols

Cell Line	Tissue Origin	Key Characteristics	Research Relevance
HeLa	Cervix (Cervical cancer)	Highly proliferative, HPV-positive, immortalized line	Used for general cytotoxicity, apoptosis, and DNA damage response studies.[41]
MCF-7	Breast (Adenocarcinoma)	Estrogen receptor-positive (ER+), hormone responsive	Ideal for studying hormonal effects, breast cancer therapies, and apoptosis pathways.[42]
A549	Lung (Adenocarcinoma)	Non-small cell lung cancer (NSCLC) with wild-type p53 shows heightened sensitivity to oxidative stress.	Frequently used in apoptosis, antioxidant, and anti-metastatic evaluations.[43]
HT-29	Colon (Adenocarcinoma)	Moderately differentiated, p53 mutant, COX-2 overexpressing	Useful for colorectal cancer, inflammation, and differentiation studies.[44]
HCT116	Colon (Carcinoma)	p53 wild type, microsatellite instability, highly proliferative	Model for cell cycle, DNA repair, and apoptosis-related pathways.[45]
PC3	Prostate (Carcinoma)	Androgen receptor-negative, highly metastatic	Suitable for hormone-independent prostate cancer and anti-migratory studies.[46]
DU145	Prostate (Carcinoma)	Moderate metastatic potential, p53 mutant	Used for comparison with PC3, studies on prostate cancer resistance.[47]
SKOV-3	Ovary (Ovarian carcinoma)	Estrogen receptor-positive, multidrug resistance potential	Used in drug resistance, apoptosis, and polyphenol sensitivity screening.[48]
U87	Brain (Glioblastoma)	High-grade glioma, aggressive, adherent morphology	Model for brain tumors, blood-brain barrier, and cytotoxicity of natural compounds.[49]
HepG2	Liver (Hepatocellular carcinoma)	p53 wild type, metabolically active hepatocyte-like cells	Common for hepatotoxicity, antioxidant, and metabolic studies.[50]
T47D	Breast (Ductal carcinoma)	ER+, PR+, p53 mutant, epithelial morphology	Used alongside MCF-7 for hormone-responsive cancer screening.[51]
Caco-2	Colon (Adenocarcinoma)	Differentiates into enterocyte-like cells, forms tight junctions	Used in drug absorption, intestinal permeability, and cytotoxicity assessments.[52,53]

Table 3. *In Vitro* Assays for Apoptosis and Cell Cycle Analysis

Assay	Principle	Purpose	Output/Readout
MTT/XTT Assay	based on how mitochondrial enzymes in living cells convert tetrazolium salts (MTT or XTT) to coloured formazan	Evaluates the proliferation and vitality of cells after treatment.	Absorbance values indicating % of viable cells (colorimetric).[53]
Annexin V-FITC/PI Staining	Phosphatidylserine (early apoptosis) is bound by Annexin V, and PI stains the DNA of late apoptotic and necrotic cells.	Detects and distinguishes early and late apoptotic cells	Flow cytometry or fluorescence microscopy quantification.[54]
Caspase Activity Assay	Measures enzymatic cleavage of specific caspase substrates (e.g., caspase-3, -8, -9) to generate fluorescent or colorimetric signals	Assesses activation of intrinsic and extrinsic apoptotic pathways	Relative fluorescence or absorbance indicating caspase activation.[55]
Flow Cytometry for Cell Cycle	DNA-binding dyes (e.g., PI) used to quantify total DNA content per cell	Analyzes cell cycle phases (G_0/G_1, S, G_2/M) to detect arrest.	Percentage of cells in each phase (G_1, S, G_2/M).[56]
Western Blotting	Protein separation and detection using antibodies against target proteins	Detects apoptosis/cell cycle regulatory proteins (e.g., Bcl-2, Bax, p53, caspases, cyclins)	Protein expression levels (band intensity).[57]
RT-PCR/qPCR	Amplifies specific mRNA transcripts using reverse transcription and real-time detection	Measures gene expression changes related to apoptosis or cell cycle	Fold change in mRNA expression of genes like p21, Bcl-2, CDK1, etc.[58]

Table 4. Enhanced Efficacy and Reduced Toxicity of Flavonoid/Polyphenol-Based Combination Therapies in Cancer

Flavonoid/ Polyphenol	Combined With	Cancer Type	Enhanced Efficacy	Reduced Toxicity	Ref
Quercetin	Cisplatin	Ovarian, Lung Cancer	↑Apoptosis via mitochondrial pathway ↓ MDR protein activity	↓ Nephrotoxicity by antioxidant protection	89
Curcumin	Doxorubicin	Breast Cancer	Inhibited NF-κB and PI3K/Akt ↓ Apoptotic signaling	↓ Cardiotoxicity	88
Resveratrol	5-FU	Colorectal Cancer	↑S-phase arrest and caspase activation	↓ Gastrointestinal mucosal damage	92
Genistein	Radiotherapy	Prostate, Pancreatic Cancer	↑Radiosensitization by cell cycle arrest and DNA damage	↓ Inflammation and fibrosis in normal tissues	91

quercetin upregulated the CDK inhibitor p21^CIP1/WAF1, which bound to and suppressed the cyclin/B1–Cdc2 complex, while notable p53-independent growth suppression was observed. The flavonoid also induced significant apoptosis, demonstrated by increased sub G DNA content, chromatin condensation, and DNA fragmentation, all of which were markedly reduced upon antisense-mediated p21 inhibition. Thus, quercetin was shown to execute a dual anticancer mechanism in MCF 7 cells: halting proliferation via reversible mitotic arrest and G checkpoint activation, and triggering apoptosis that is tightly linked to p21, but independent of p53 status.[61]Kaempferol significantly affects DNA integrity, apoptosis, cell cycle regulation, and proliferation in MDA-MB-231 triple-negative breast cancer (TNBC) cells through its anticancer mechanisms. The flavonol was more successful in inhibiting cell proliferation in TNBC MDA MB 231 cells than in ER-positive BT474 cells. Kaempferol significantly changed the distribution of cell cycles after 48 hours of treatment, boosting G -phase cells from 9.3% to 37.5% and decreasing G -phase cells from 85.5% to 51.4%, indicating a robust induction of G /M arrest. At the same time, apoptosis and DNA damage indicators were significantly increased: phosphorylated ATM, cleaved versions of caspase-9 and caspase-3, and ãH2AX, a DNA double-strand break indicator, were all upregulated. By stopping the cell cycle at the G /M checkpoint, triggering DNA damage reactions, and triggering mitochondrial-mediated apoptosis, kaempferol prevents the growth of TNBC cells. Given its selective potency in aggressive breast cancer subtypes, kaempferol shows notable potential as a therapeutic agent against TNBC.[62]In HL 60 human acute leukemia cells, structurally identical flavonoids with antioxidant qualities influence apoptosis and cell cycle progression. The study examined six flavonoids: quercetin, myricetin, kaempferol, isorhamnetin, 32 methylquercetagetin, and taxifolin. The results showed that while 32 methylquercetagetin and taxifolin had negligible effects, quercetin, myricetin, kaempferol, and isorhamnetin significantly decreased cell growth. the role of specific structural components, including the 6 hydroxyl group and the C2-C3 double bond, in mediating antiproliferative activity. Flow cytometry data showed that myricetin and quercetin were the most effective apoptosis inducers. . Myricetin, isorhamnetin, and kaempferol induced concentration- and time-dependent increases in G€ /G and S phase cells, while quercetin alone triggered G, /M arrest. Notably, antioxidant assays revealed that myricetin showed the greatest capacity to scavenge DPPH radicals, though it was less effective at reducing intracellular ROS in response to menadione stress. On the other hand, kaempferol was very effective at scavenging ROS when cells were under stress. Apart from their antioxidant capacity, small structural differences may affect flavonoids' capacity to stop the cell cycle's advancement and cause leukemia cells to undergo apoptosis.[63]

Flavanones

Naringenin, a citrus flavonoid, shows anticancer effects by inducing apoptosis and cell cycle arrest across various cancer cell lines. In HepG2 cells, it inhibits proliferation, increases p53, and induces biphasic arrest at G€ /G and G, /M phases. Apoptosis is evidenced by nuclear condensation, sub-G population increase, cytochrome c release, elevated Bax/Bcl-2 ratio, and caspase-3 activation, suggesting its potential as a liver cancer therapeutic.[64]Naringenin's anticancer actions in A431 human epidermoid carcinoma cells revealed a ROS-mediated apoptotic mechanism in conjunction with G€ /G death reduction in cell viability that is dose-dependent (p/ </ 0.01) and accompanied by apoptotic hallmarks such as DNA fragmentation and nuclear condensation. Intracellular ROS generation increased markedly (p/ </ 0.001), leading to mitochondrial membrane depolarization. Flow cytometry revealed caspase-3 activation and G€ /G cell cycle arrest, indicating that naringenin inhibits epidermoid carcinoma cell growth through oxidative stress, mitochondrial dysfunction, caspase activation, and cell cycle blockade.[65]Citrus-derived flavanones naringin and naringenin have several anticancer properties, including the capacity to regulate key cellular functions that inhibit cancer, including invasion, metastasis, angiogenesis, proliferation, apoptosis, and cell cycle arrest. By disrupting multiple signaling pathways, these flavanones produce a variety of pleiotropic effects, including regulating inflammation and cytokine production, inhibiting growth factor receptors (EGFR, VEGF), and suppressing downstream kinases (PI3K/Akt,

NF-êB, MAPKs), which results in cell cycle blockade and intrinsic and extrinsic apoptosis. In particular, they cause cytochrome c release, activate caspases-8 and -9, and upregulate pro-apoptotic proteins (Bax, Bak); these actions result in effector caspase-3, which initiates apoptosis. Additionally, via altering cell cycle regulators including p53, p21, CDKs, and cyclins, naringin and naringenin cause arrest at the G€ /G and G, /M phases. Their capacity to stop angiogenesis, invasion, and metastasis further increases their therapeutic potential.[66]

Isoflavones

Isoflavones, particularly genistein, are plant-derived polyphenolic compounds predominantly found in soybeans and legumes, and are well-known for their estrogen-like activity and strong anticancer potential. Genistein induced apoptosis, G, /M arrest, and reduced proliferation in HCT 116 (p53 wild-type) and SW 480 (p53 mutant) colon cancer cells. In HCT 116, it dose- and time-dependently increased G, /M-phase cells, downregulated CDC2 and CDC25A, and activated ATM, p53, p21^WAF1/CIP1, and GADD45á. Using flow cytometry with Annexin V/PI labelling to validate enhanced apoptosis, especially in p53-competent cells, the p53-dependency of this response was shown. Furthermore, real-time PCR and Western blot investigations revealed that genistein markedly downregulated anti-apoptotic factors like Bcl-2 while upregulating cell cycle checkpoint genes including CDKN1B and BRCA1/2. This implies that a crucial regulatory network mediating the chemopreventive effect of genistein is the ATM/p53–p21 axis. These findings illustrate that genistein can exert dual anticancer effects in colon cancer halting proliferation through G, /M arrest and triggering apoptosis via the DNA damage response pathway, with efficacy influenced by p53 status.[67] In human ovarian cancer cells, genistein induces DNA damage, G, /M cell cycle arrest, and death via triggering DNA damage checkpoint mechanisms (HO 8910). G, /M phase arrest was enforced by phosphorylating and activating ATM and ATR, which in turn activated checkpoint kinases Chk2 and Chk1. This, in turn, phosphorylated and inactivated Cdc25C/A phosphatases and inhibited Cdc2. Genistein promoted apoptosis by downregulating phosphorylated Akt, enhancing p53 activation,

and reducing Bcl-2/Bax and Bcl-xL/Bax ratios. According to these results, genistein uses DNA damage response systems, particularly the ATM–Chk2–Cdc25 and ATR–Chk1–Cdc25 pathways, to halt the advancement of the cell cycle during G, /M. When the damage is irreversible, it triggers mitochondrial-mediated death. This dual action reinforces genistein's antiproliferative and pro-apoptotic potential in ovarian cancer via genotoxic stress.[68]Numerous cell cycle modulation and cell death pathways were revealed by the biological activity of novel polysaccharide complexes and genistein derivatives in MCF 7 breast cancer cells and HL 60 leukemia cells. While XG G shown no antiproliferative activity, two analogues, IFG 027 and IFG 043, as well as the complex SPG G, demonstrated antiproliferative effects that were on par with or even stronger than genistein. Interestingly, the analogues IFG 027 and IFG 043 actually reduced cell accumulation in G, /M, indicating different effects on cell cycle regulation from genistein and the SPG G complex, which caused G, /M phase arrest. Both genistein and its derivatives triggered caspase-3, but SPG G encouraged apoptosis that was independent of caspase 3. Apoptotic studies revealed that SPG G, IFG 027, and IFG 043 produced phosphatidylserine externalisation and decreased mitochondrial membrane potential, indicating induction of apoptosis. However, the absence of typical apoptotic markers and the production of acidic vesicular organelles by XG G suggested either autophagic or non-apoptotic cell death. These findings illustrate that structural modifications of genistein can lead to distinct anticancer activities, from conventional apoptosis to alternative death pathways, and emphasize the potential of tailored derivatives for targeted cancer therapeutics.[69]Several flavonoids, including quercetin, kaempferol, apigenin, luteolin, naringenin, and genistein, exhibit strong anticancer effects by inducing cell cycle arrest and apoptosis in various *in vitro* cancer models such as MCF-7, MDA-MB-231, HT-29, HCT116, A549, PC3, DU145, HepG2, HeLa, and HL-60. These effects are dose-dependent, typically observed between 10–100 µM. For example, quercetin causes G2/M arrest and apoptosis in MCF-7 cells at ~90 µM, while naringenin induces G0/G1 arrest and mitochondrial death in HepG2 cells at 50 µM. Genistein (30–60 µM) leads to G2/M arrest and

caspase-mediated apoptosis in colon and ovarian cancer cells.[70]Flavonoids modulate multiple apoptotic proteins, activating initiator (caspase-8, -9) and executioner (caspase-3) caspases. They downregulate anti-apoptotic proteins (Bcl-2, Bcl-xL) and upregulate pro-apoptotic factors (Bax, Bak, cytochrome c). Additionally, flavonoids influence p53 directly or via ATM/ATR, increasing p21^WAF1/CIP1 and p27^KIP1 transcription, thereby inhibiting cyclin–CDK complexes and inducing cell cycle arrest at G0/G1 or G2/M depending on cell type and flavonoid. For example, apigenin and luteolin suppress cyclin D1/CDK4/6 to enforce G1 arrest, while kaempferol and genistein downregulate cyclin B1/CDK1 to cause G2/M arrest.[71]Flavonoids disrupt survival signaling cascades such PI3K/Akt, NF-êB, and MAPK/ERK pathways in addition to these traditional apoptotic and cell cycle routes. Cell survival is decreased when Akt phosphorylation is suppressed, while pro-inflammatory and anti-apoptotic gene expression is decreased when NF-êB transcriptional activity is inhibited. The regulation of MAPK by flavonoids influences the ratio of apoptosis to proliferation. These pleiotropic molecular effects not only result in cytotoxicity and growth arrest, but also sensitize cancer cells to chemotherapeutic agents, making flavonoids potential candidates for combination therapies.[72]Taken together, the evidence underscores that flavonoids act as multifunctional anticancer agents, capable of targeting key hallmarks of cancer such as uncontrolled proliferation, evasion of apoptosis, and dysregulated cell cycle progression. Their attractiveness as natural substances for cancer chemoprevention and adjuvant therapy approaches is increased by their selective toxicity to cancer cells and minimal toxicity to healthy cells.

Polyphenols' Role in Cell Cycle Arrest and Apoptosis

Resveratrol, curcumin, gallic acid, and ellagic acid are polyphenols with strong anticancer properties, primarily through apoptosis induction and cell cycle regulation. Found in fruits, vegetables, spices, and medicinal plants, these compounds target key survival pathways in cancer cells.

Resveratrol, a stilbene in berries and grapes, induces apoptosis via mitochondrial and death receptor pathways by activating caspases-3, -8, and -9, increasing Bax, and releasing cytochrome c. It also modulates cyclin A, cyclin B1, CDK1, p21, and enhances p53 signaling, leading to S or G2/M phase arrest in prostate cell lines like PZ-HPV-7, LNCaP, and PC-3. Resveratrol significantly increased apoptosis and decreased proliferation in cancer cell lines, which led to a buildup of cells in the G€ /G phase over time. A higher Bax/Bcl-2 ratio suggested the presence of the intrinsic apoptotic pathway, and both LNCaP and PC-3 cells experienced apoptosis as a result of caspase-9 and -3 activation. Resveratrol has been demonstrated to decrease CDK4 expression, cyclin D1/CDK4 kinase activity, and cyclins D1 and E in LNCaP cells. In contrast, both PC-3 and LNCaP cells displayed decreased levels of cyclin B and Cdk1, as well as decreased cyclin B/Cdk1 kinase activity. Additionally, resveratrol increased levels of p53, p21, and p27 but only in LNCaP cells suggesting a more robust G€ /G checkpoint control in androgen-sensitive cells. Together, these molecular changes explain the observed cell cycle arrest and apoptosis induction, demonstrating that resveratrol's anticancer actions entail unique pathways impacted by p53-related pathway activation and androgen sensitivity.[73]HCT 116 and Caco 2 colorectal cancer cells experienced considerable G /S phase cell cycle arrest and caspase-dependent death as a result of resveratrol's antiproliferative and pro-apoptotic actions. Significant cell accumulation at the G /S checkpoint and a marked decrease in cell viability were the outcomes of treatment with increasing resveratrol concentrations (10-100/ μM for 24 hours). Additionally, there was a dose-dependent decrease in important cell cycle regulators like cyclin D1, CDK2, CDK4, PCNA, and an increase in p21 expression. Annexin V/PI staining and Western blot confirmed apoptosis via increased cleaved caspase-9, caspase-7, and PARP, indicating activation of the intrinsic pathway. Notably, Caco 2 cells exhibited greater sensitivity to resveratrol compared to HCT 116 cells, demonstrating more pronounced effects on both proliferation inhibition and cell cycle arrest. These findings demonstrate how strongly resveratrol inhibits colorectal cancer cell growth by concurrently targeting the caspase and cyclin CDK pathways, confirming its potential as a chemopreventive medication against colon neoplasia.[74]Resveratrol's effects on human neuroblastoma in a mouse xenograft model include

both *in vitro* cytotoxicity and tumour growth suppression. Resveratrol induced G€ /G   arrest in neuroblastoma cells by upregulating p21 and downregulating CDK2, CDK4, and cyclin D1. Apoptosis was marked by sub-G   accumulation, chromatin condensation, DNA fragmentation, caspase-9/3 activation, and PARP cleavage. These cellular findings were supported by *in vivo* studies showing that resveratrol administration significantly suppressed tumor growth in immunodeficient mice bearing neuroblastoma xenografts, without inducing major systemic toxicity . Together, the data highlight resveratrol's dual action hindering cell cycle progression and inducing apoptosis as key contributors to its antitumor efficacy against neuroblastoma both in cell culture and animal models.[75] Resveratrol efficiently and dose-dependently suppressed cell proliferation (50-250 μM), and its cytotoxic effects on 4T1 murine breast cancer cells demonstrated that it was more harmful to cancer cells than to normal cells. Following resveratrol administration, transcriptome profiling identified 330 differentially expressed genes related to apoptosis and cell cycle regulation, of which 103 were upregulated and 227 were downregulated. Gene expression and pathway analysis revealed that resveratrol induced S-phase arrest, which led to a rise in S-phase cells and a fall in G€ /G   phase cells. Resveratrol induced strong anti-proliferative effects in breast and bladder cancer cells by causing S and G   phase arrest, respectively, and promoting apoptosis. It increased early and late apoptotic populations, activated caspases 9 and 3, cleaved PARP, downregulated p-Rb, cyclin D1, CDK4, and upregulated p21.[76]At a dose of 20 mg/kg/day, resveratrol also significantly suppressed tumour growth in T24 xenografts by reducing production of VEGF and FGF 2, decreasing Akt phosphorylation while increasing p38 MAPK activity, and modulating important signaling pathways. According to these findings, resveratrol is a strong contender for bladder cancer chemoprevention since it exhibits anticancer properties through a complex mechanism that includes cell cycle arrest, mitochondrial-mediated apoptosis, and inhibition of survival and angiogenic pathways.[77]

Curcumin, derived from turmeric (*Curcuma longa*), exerts pleiotropic effects by inhibiting the NF-êB, PI3K/Akt, and STAT3 pathways, leading to apoptosis and G2/M arrest. It suppresses cyclin D1, Bcl-2, and survivin, while enhancing cleaved PARP and caspase activation. Curcumin's anticancer actions on BxPC 3 human pancreatic cancer cells at a physiologically appropriate dose of 2.5 ìM showed a DNA damage-mediated mechanism that causes apoptosis and G, /M cell cycle arrest. After 24 hours of treatment, increased phosphorylation of ATM, Chk1, and H2A.X indicated DNA damage response activation, while reduced DNA polymerase-â suggested impaired DNA repair. This signaling cascade resulted in Cdc25C phosphorylation (Ser 216) and subsequent downregulation of cyclin B1/CDK1 complexes, effectively enforcing G, /M arrest; these effects were confirmed by flow cytometry. Concomitant apoptotic induction was evidenced by caspase-3 activation and PARP cleavage, selectively affecting cancerous cells but sparing normal HPDE 6 pancreatic ductal epithelial cells. Importantly, siRNA reduction of ATM or Chk1 abolished curcumin's effects by preventing apoptosis, G, /M arrest, and phosphorylation of ATM/Chk1/Cdc25C. The importance of the ATM–Chk1–Cdc25C axis in curcumin's anticancer properties is shown by this.[78]Curcumin inhibited proliferation of PANC-1 and BxPC-3 cells in a dose- and time-dependent manner, induced apoptosis, and caused G, /M phase arrest, as shown by CCK-8 and flow cytometry analyses. Immunofluorescence and Western blot investigations showed autophagosome production and elevation of LC3-II and Bax, while Bcl-2 was downregulated, indicating a link between autophagy and apoptosis. A strong inverse correlation between the LC3-II/Bax ratio and cell viability implied that both autophagy and apoptosis contribute cooperatively to curcumin's cytotoxic effects. These findings position curcumin as a multitargeted anticancer agent that simultaneously activates autophagic pathways, mitochondrial apoptosis, and G, /M cell cycle arrest, particularly at higher doses underscoring its potential for therapeutic development in pancreatic cancer.[79]Curcumin's antiproliferative actions in human osteosarcoma (HOS) cells show that, at an IC... € of roughly 4/ ìg/mL, it causes sequential cell cycle arrest and death. Flow cytometry analysis revealed that curcumin treatment results in biphasic arrest at both G   /S and G, /M phases, followed by

extensive apoptotic cell death, characterized by nuclear fragmentation and DNA degradation. Mechanistically, cyclin B1 (G, /M) and cyclin D1 (G /S) and CDC2 (CDK1) were downregulated in conjunction with these cell cycle blocks. Apoptosis was facilitated by caspase 3 activation and PARP cleavage.[80]

Ellagic acid, found in pomegranates and berries, induces G€/G arrest by inhibiting cyclin D1 and upregulating p21 and p27. It generates ROS, disrupts mitochondrial potential, and triggers apoptosis in MCF-7 cells via the TGF-â/Smad3 pathway. EA reduces cell proliferation by downregulating cyclins A2 and E2 and overexpressing CDK inhibitors such p21, CIP1, p15, and p19. Together, these inhibitors were able to eliminate cyclin CDK complexes and halt the cell cycle's progression. Using cDNA microarray analysis after 6, 12, and 24-hour EA exposure, the authors observed major transcriptional shifts with over 4,700 genes altered and subsequently validated 16 genes within the TGF â/Smad signaling axis via RT PCR and Western blotting. Activation of this pathway through Smad3 phosphorylation emerged as the core mechanism: EA enhanced TGF â signaling, which in turn increased expression of CDK inhibitors while suppressing cyclins, effectively enforcing G€/G arrest. Additionally, this cell cycle blockade correlated with induction of apoptosis, positioning TGF â/Smad3 as a critical effector of EA's anticancer activity in breast cancer cells.[81] The combined effects of ellagic acid, quercetin, and resveratrol polyphenols commonly found in muscadine grapes on MOLT 4 human leukemia cells and discovered notable synergistic pro-apoptotic and antiproliferative activities. While combinations greatly increased caspase 3 activation and decreased cell proliferation more than the sum of their individual effects, each drug alone caused transitory cell cycle arrest. Isobolographic analysis confirmed synergy with combination indices of 0.64 (ellagic acid + resveratrol) and 0.68 (quercetin + resveratrol).[82] Gallic acid, present in tea, grapes, and gallnuts, induces G, /M arrest and mitochondrial apoptosis via ROS, Bax upregulation, and Bcl-2 downregulation. In DU145 prostate cancer cells, it reduced proliferation dose- and time-dependently by activating ATM, H2A.X, and Chk2, indicating a DNA damage response. G, /M phase arrest resulted from this activation's inhibition of Cdc25A/C phosphatases' and Cdc2 kinase's phosphorylation. The upstream ATM-Chk2 signaling pathway was validated when caffeine, an ATM inhibitor, abolished these effects. Additionally, gallic acid cleaved PARP, caspase-9, and caspase-3, suggesting that it employed mitochondrial pathways to trigger apoptosis. It's interesting to note that apoptosis continued even after pan-caspase inhibition, which raises the prospect of caspase-independent processes.[83]

Gallic acid's antiproliferative and pro-apoptotic effects on HL 60 human promyelocytic leukemia cells led to G€/G cell cycle arrest and a dose- and time-dependent reduction in cell viability. Mechanistically, gallic acid elevated levels of CDK inhibitors p21 and p27 while concurrently suppressing cyclins D and E, effectively halting cell cycle progression. Additionally, the compound triggered DNA damage, evidenced by DAPI staining and comet assay, and induced mitochondrial dysfunction with reduced membrane potential and increased intracellular Ca^2z. The process of apoptosis included both mitochondria-dependent mechanisms such as the release of cytochrome c, AIF, and endonuclease G, as well as death receptor pathways such as Fas/FasL, caspase 8, and Bid. These changes were verified at the protein and mRNA levels using real-time PCR and Western blotting.[84] Together, these polyphenols exert multi-targeted anticancer effects, modulating redox balance, gene expression, and cell signaling to inhibit cancer cell proliferation and enhance apoptotic responses. Their natural origin, low toxicity, and broad spectrum of activity position them as attractive candidates for both preventive and therapeutic strategies in oncology. According to mechanistic study, these drugs work by blocking cyclins and cyclin-dependent kinases, activating intrinsic mitochondrial apoptotic pathways, and changing key regulators like p53, Bax, Bcl-2, and caspases. Depending on the kind of cancer cell, resveratrol, for example, induces caspase-mediated apoptosis through the ATM/Chk2 or PI3K/Akt suppression pathways, leading to either G1/S or G2/M phase arrest. Curcumin exhibits similar effects, downregulating cyclin B1 and CDK1 and activating the ATM/Chk1 axis to cause cell cycle arrest and death.[85] Moreover, polyphenols can cooperate with conventional chemotherapy medications such as doxorubicin, cisplatin, and

5-fluorouracil. These combinations often enhance drug efficacy, reduce resistance, and lower the effective therapeutic dose, thereby minimizing systemic toxicity. Studies have demonstrated that co-treatment with polyphenols not only augments cancer cell death but also modulates drug transporters and survival signaling pathways, improving overall therapeutic outcomes.[86] Notably, polyphenols exhibit a degree of selectivity, exerting cytotoxic effects preferentially on malignant cells while sparing normal cells. This differential action is attributed to altered redox status, mitochondrial integrity, and proliferation rates in cancer cells. Such selectivity makes polyphenols attractive candidates for integrative cancer therapy, supporting their continued investigation in preclinical and clinical settings.[87]

DISCUSSION

Synergistic and Combinatorial Effects

The combination of flavonoids and polyphenols with conventional cancer therapies has gained significant attention for enhancing therapeutic efficacy and minimizing side effects. Combining flavonoids or polyphenols with chemotherapeutic drugs like doxorubicin, 5-fluorouracil, and cisplatin has been shown in numerous trials to provide synergistic effects, such as increased apoptosis, decreased drug resistance, and greater susceptibility of cancer cells.[88] Quercetin and curcumin sensitize cancer cells to cisplatin by downregulating Bcl-2 and upregulating Bax and caspases.[89] Similarly, by regulating oxidative stress and blocking survival signaling pathways including NF-êB and PI3K/Akt, resveratrol increases the cytotoxic potential of doxorubicin.[90] Flavonoids such as genistein and luteolin have been shown to function as radiosensitizers in the setting of radiation therapy by increasing DNA damage, blocking DNA repair processes, and encouraging cell cycle arrest. This not only boosts the radiation response in tumor cells but also protects normal cells through their antioxidant properties, thereby improving the therapeutic window.[91] Furthermore, combining multiple phytochemicals such as ellagic acid, gallic acid, and resveratrol has demonstrated additive or even synergistic effects in various cancer models. These combinations enhance cellular stress, disrupt

multiple signaling pathways, and offer a broader spectrum of anticancer action. The combinatorial use of polyphenols with existing therapies thus holds promise for overcoming resistance, reducing toxicity, and achieving more effective cancer management, paving the way for integrative and personalized oncology strategies. Numerous studies have documented the enhanced efficacy and reduced toxicity of combining flavonoids and polyphenols with conventional cancer therapies. These organic chemicals not only improve the therapeutic efficacy, but they also reduce the unfavourable side effects typically associated with radiation and chemotherapy. (Table.4)

Limitations and Challenges

Despite the promising anticancer properties of polyphenols and flavonoids demonstrated in numerous preclinical studies, several limitations and challenges hinder their full therapeutic translation. One of the primary concerns is their poor bioavailability and metabolic stability. Many polyphenols, including curcumin and quercetin, undergo rapid metabolism and systemic elimination, resulting in low plasma concentrations that may not replicate the effects observed *in vitro*. Additionally, the discrepancy between *in vitro*, *in vivo*, and clinical outcomes poses a major challenge. While cell culture models allow for mechanistic insights, they fail to fully mimic the complexity of human tumors, such as tumor microenvironment interactions, immune responses, and pharmacokinetics. Another significant hurdle is the lack of standardized screening protocols. Different studies often use varying cell lines, dosages, time points, and assay methods, making it difficult to compare or replicate findings across research groups. Furthermore, the absence of large-scale, head-to-head comparative studies on flavonoids and polyphenols limits the ability to rank their efficacy, safety profiles, and mechanisms systematically. The use of uncharacterized plant extracts in some studies also complicates the identification of active constituents and their individual roles. To overcome these obstacles, standardized *in vitro* and *in vivo* models, sophisticated delivery methods to boost bioavailability, and well-planned clinical trials are needed. These problems need to be fixed if polyphenols and flavonoids are to reach their full therapeutic potential in the treatment of cancer.

Future Perspectives

Future research into the anticancer potential of flavonoids and polyphenols is poised to benefit greatly from advances in nanotechnology and targeted delivery systems. Nanocarriers like as liposomes, solid lipid nanoparticles, and polymeric micelles can assist overcome one of the main barriers to the widespread use of these treatments by dramatically increasing their bioavailability, stability, and tumor-specific accumulation. Furthermore, a strong platform for deciphering the intricate molecular mechanisms impacted by polyphenols and locating biomarkers of response is provided by the combination of omics technologies like as transcriptomics, proteomics, and metabolomics. These tools can facilitate the development of precision oncology strategies, where patient-specific flavonoid or polyphenol responsiveness is predicted based on molecular signatures. There is also increasing interest in tailoring polyphenol-based interventions as part of personalized cancer therapy, leveraging individual metabolic profiles, genetic predispositions, and tumor characteristics. Such approaches could allow for optimized combination therapies, lower toxicity, and improved patient outcomes. However, the realization of these strategies requires robust *in vivo* validation in animal models and, more importantly, well-structured clinical trials to establish safety, efficacy, and dosage guidelines. Presently, a limited number of clinical studies have explored these compounds in isolation or adjunctively, and their outcomes underscore the need for larger, controlled trials with standardized endpoints. As interdisciplinary collaboration grows between pharmacologists, oncologists, and systems biologists, flavonoid and polyphenol research holds great promise for next-generation, personalized anticancer therapies.

CONCLUSION

Many flavonoids and polyphenols found in dietary and medicinal plants influence essential biological processes such cell cycle arrest and apoptosis induction, which has potent anticancer effects. These natural chemicals have shown strong dose-dependent antiproliferative effects on a variety of human cancer cell lines, including those from the liver, ovary, breast, colon, lung, and leukemia. According to their mechanism of action, they interfere with important pathways like PI3K/Akt, NF-êB, and MAPKs, control important signaling molecules like Bcl-2 family proteins, caspases, cyclins, CDKs, and tumour suppressors like p53 and p21, and work through both intrinsic and extrinsic apoptotic pathways. Their capacity to halt tumor cell progression and promote programmed cell death underscores their potential as promising agents for cancer chemoprevention and therapy. Reaffirming their significance, flavonoids and polyphenols represent a non-toxic, readily available, and multi-targeted approach to combating cancer. Their selective action on malignant cells and synergistic effects with conventional therapies highlight their utility as adjunct treatments in modern oncology. However, despite encouraging *in vitro* findings, the translation of these compounds into clinical applications remains limited due to challenges related to bioavailability, metabolism, and mechanistic complexity. Therefore, further in-depth mechanistic studies, pharmacokinetic evaluations, and clinical investigations are essential to validate their therapeutic efficacy and optimize their use in evidence-based integrative cancer care and treatment strategies.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

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Author's Contribution

Binoy Varghese Cheriyan: Visualization, Supervision, Project Adminstration; Kaniga Pandi: Writing – Original Draft; Dharshan murali, Nesayan Boobalan, Lini priyadharshini christopher jebaraj : Funding Acquisition, Resources; Jeevitha Harikrishnan , Deepshikaa Kannan :Data Collection, Analysis; Shamyuktha Munuswamy , Shagul Hameed Manaksha : Analysis.

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