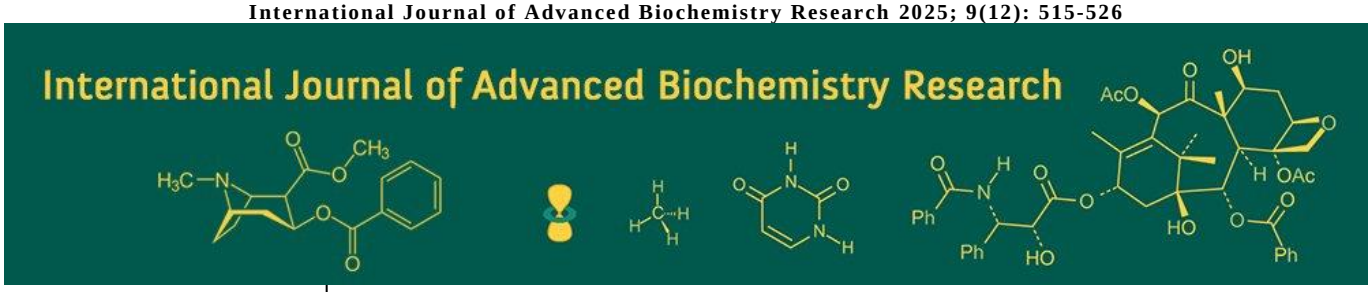

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(12): 515-526
www.biochemjournal.com
Received: 08-09-2025
Accepted: 13-10-2025

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MTT-based evaluation of plant-derived phenolics: A comprehensive review of their antiproliferative effects in cancer models

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i12g.6554

Abstract
The review offers an extensive overview of the antiproliferative properties of plant-based phenolic compounds on the system of cancer research with MTT assay as the main evaluation tool. Phenolic acids, flavonoids, stilbestenes, tannins, and phenolic compounds are largely known as strong antioxidants and cytotoxic agents with numerous molecular mechanisms of cancer progression. The MTT assay has been considered as one of the most effective, cost effective as well as extensively used colorimetric procedures in the evaluation of cell viability, metabolic activity and cytotoxicity brought about by drugs. It allows the quantitative determination of the activity of dehydrogenase in mitochondria which is an indirect yet good measure of cell proliferation and viability. This review presents findings of recent studies that show in vitro that plant-derived phenolics can inhibit the growth of cancer cells by inducing apoptosis, arresting cell cycle, regulating the production of oxidative stress and inhibiting oncogenic signaling pathways. The particular attention is given to the comparison of the antiproliferative activity of various phenolic classes on a variety of cancer cell lines such as breast, colon, liver, and lung cancers. Dose response relationships, IC50 values and variation in sensitivity between the cancer models are also addressed in the review. In addition, the paper has assessed methodological issues associated with MTT assay such as the effect of solvents, incubation period, stability of phenolic compounds and any interference with the assay. Difficulties like overestimation or underestimation of cytotoxicity because of the color of a compound, its solubility or redox potential are discussed in a very significant manner. In general, this review demonstrates the necessity to combine MTT-based tests with other biochemical and molecular methods in an attempt to have a more precise overview of the cytotoxicity caused by phenolics. The results highlight the therapeutic role of the plant phenolics as potential anticancer agents with promise and advocate further studies to streamline the applications in preclinical cancer models.

Keywords: Plant-derived phenolics, MTT assay, Antiproliferative activity, Cancer cell models, Cytotoxicity evaluation, Phenolic compounds

1. Introduction
Cancer is a serious killer disease associated with the global death rate as despite the existence of numerous chemotherapeutic agents, their effectiveness on cancer treatment frequently depends on the development of drug-resistant cells, which negatively influence the survival of patients. As a result, there is an ongoing trend of discovering natural anticancer agents by the researchers, and this could result in the discovery of new therapeutic agents with enhanced effectiveness and fewer side effects [1]. Plant phenolics are secondary metabolites containing a phenol ring-structure that are significant in regulating vital processes in plants. They are widely distributed in nature and can be found as single compounds, in soluble form, or polymerized into large molecules named tannins. This broad range of compounds from various sources exhibit anticancer properties and address different cellular targets and regulatory pathways [2, 3]. Phenolic compounds have been shown to inhibit cancer cell proliferation in a wide variety of animal and human cancer cell lines. MTT (thiazolyl blue tetrazolium bromide) assays are commonly employed to evaluate candidate compounds as anticancer agents by estimating their effect on cell viability across a range of concentrations. The principal of the MTT assay relies on the bio-reduction of MTT to formazan by living cells. Formazan can be solubilized and quantified by measuring absorption. Growth

inhibition can be expressed as the inhibitory concentration of 50% (IC_{50}) value or as percentages of growth inhibition, depending on the culture medium used and the effect on cell morphology [4].

2. Methodological Foundations of MTT Assays in Cancer Research

In cancer research, the MTT assay is commonly employed to assess cell viability, proliferation, or cytotoxicity. MIT-based evaluation of plant-derived phenolics for their antiproliferative properties warrants consideration of the assay's methodological foundations. Cell-viability determination via MTT is based on the capacity of viable cells to reduce the yellow tetrazolium MTT salt to an insoluble purple formazan that can be spectrophotometrically quantified [5]. MTT assays exhibit robust two-parameter characteristics: sensitivity and linearity of dose-response curves, which facilitate modeling of structure-activity relationships and permit direct comparison of independent datasets. The dynamic range is large in general and major changes in the absorbance can be noticed after the treatment with a vast range of agents. Concerning the physiologically relevant biologically active doses, the representative concentrations which undergo limited bioavailability in vivo [6], and consequently cause an anticancer response in an isolated cell/organism environment alone, are suitable [6, 7].

2.1. Principle and Sensitivity of MTT Assay

MTT assay (3-[4, 5-dimethyl thiazol-2-yl]-2, 5-diphenyl tetrazolium bromide is a colorimetric technique that is widely applied to study the viability and proliferation of cells [5]. The assay is based on the reduction of MTT to formazan crystals by viable cells and the resulting optical density (OD) at a wavelength of 570 nm used as an indirect measure of metabolic activity, hence the degree of cell viability [8]. MTT assay has especially taken center stage in the research on plant-produced phenolics because most of them are antiproliferative agents. The effect of the phenolic treatment on the cell viability should be calculated, as some of the phenolic compounds have cytotoxic properties, as well. The MTT assay will help researchers to determine such cytotoxicity events, and choose a certain concentration of phenolic reaction, which preserves optimal cell viability, then assess other phenolic properties [8, 9]. The analysis of MTT assay shows that this test has good sensitivity and can be used to detect viable cells in a broad concentration range, making it possible to use the representative ranges of phenolic compounds that are selective to the different types of cancer. The MTT assay has been used in numerous studies examining the antiproliferative effects of plant-derived phenolics and has determined the corresponding dosage and time-course of the assay on cancer cells [10]. The sensitivity of MTT assay is influenced by diverse parameters, such as the cell line type, initial cell density, medium of cell culture, incubation period available to form formazan, and the complementary components of the treated samples such as the solvent of phenolic solubilization. The antiproliferative effects of plant-derived phenolics on different cell lines have been studied, which have indicated different ranges of concentration that display good viability, Figure 1 [10, 11].

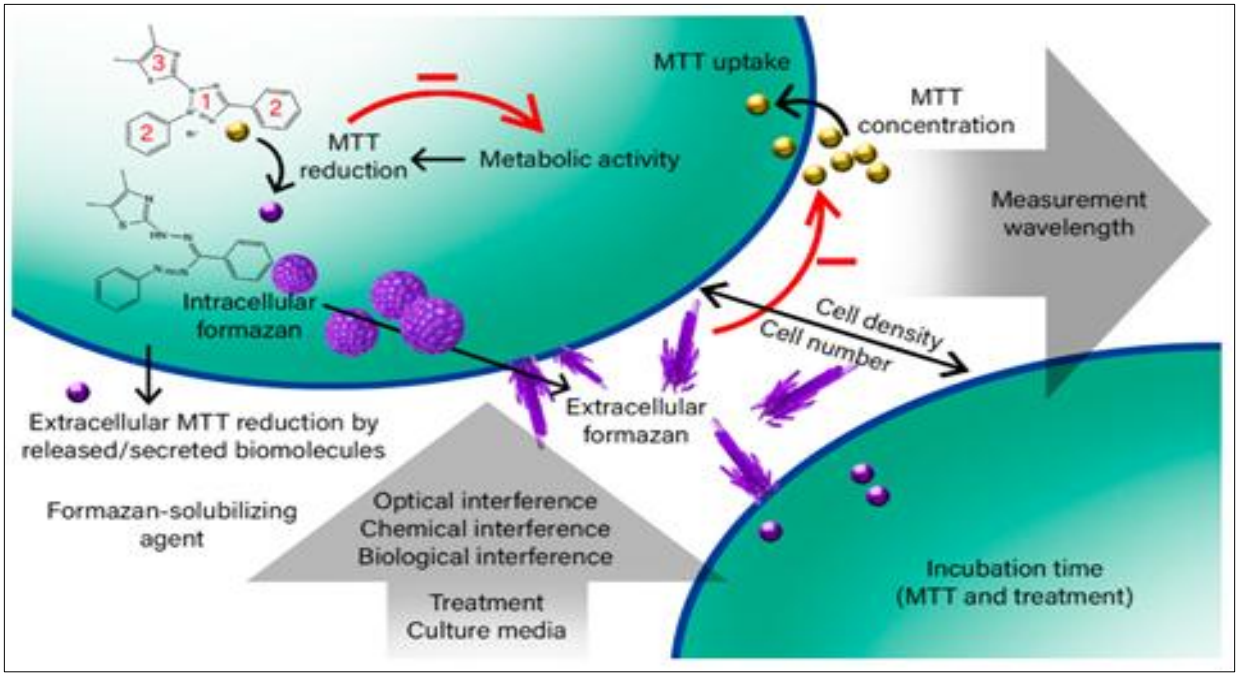

Fig 1: The ultimate optical density (OD) of the MTT assay depends on a variety of factors, such as the concentration and uptake of MTT reagent into the cell, the activity of the cell and its number, and the time of formazan crystals formation. The measurement can also be affected by other variables including culture media effects, cell density, toxic treatments which slow down metabolism and chemical or optical interference by media, treatments, or released cellular components. The incubation time using MTT and end optical reading are important. The chemical formulas of MTT and formazan are demonstrated on the cell.

2.2. Experimental Design Considerations

The method of experimental design and performance of the phenolic MTT studies should follow a number of principles to best extract the information within a specific dataset, clearly present the findings and guarantee cross-study interferences. The selection of cell lines and treatment regimens in biomarkers based on a specific context of cancer, and that has biological relevance to the phenolic

compounds of interest, is imperative. The selection of cell lines in MTT evaluations of plant-derived phytochemicals remains underexplored despite the substantial effort directed toward the assessment of these materials, and candidate compounds have been reported to exhibit highly selective activity against specific cancer types [12, 13]. The application of constituent concentrations that bracket those encountered physiologically following oral administration of whole-plant products considerably enhances the biological relevance of the findings obtained. Proliferation studies have indicated that many flavonoids and phenolic acids reach mid- to low-micromolar concentrations in human plasma following the ingestion of plant-derived materials such as cocoa and tea, whereas the systemic concentrations of stilbenes and lignans remain below 100 nm [14]. The precise brackets applied will vary according to the vehicle and delivery approach, and establishing a suitable criterion for the range investigated is therefore recommended to assist in the interpretation of phenolic evaluations [15, 16]. Viability readouts may be determined as early as 2 h post-treatment for a number of phenolics and cancer types, although exposure times extending to 24 h offer greater coverage of the readily available structural space. Another consideration is the dispensing of the dissolution solvents. The bioactivity of some phytochemicals, such as vanillin, 2-hydroxybenzaldehyde, and 4-hydroxybenzoic acid, is significantly decreased at the point of provision in co-solvents or solubilizing agents, like ethanol, dimethyl sulfoxide, and polyethylene glycol 400, and demonstrates the possible effect of formulation approaches on observed activity [17, 18]. The solvent should only be added in the first solution during the phenolic testing. These concerns can be further addressed by long incubation times in the assay, but reduced solvent concentrations are preferential in situations where the readout of an early assay is still required [19, 20].

2.3. Limitations and Artifacts in Phenolic Evaluations

Plant phenolics are common in the environment, and they have a variety of effects on the systems of the cell. The possible health effects of phenolics and other polyphenols have been of much concern, and their anticancer effects are still poorly comprehended. A recent study of 298 stilbenes and flavonoids and other related compounds in assessing their cytotoxicity effect on four different types of cancer identified three out of sixteen stilbenes and four out of fifty-five flavonoids that were active [21, 22]. Cancer has been a worldwide health menace that has afflicted millions of individuals worldwide and cost billions of dollars in health care expenditure. Breast cancer is the prevalent cancer and the rate of deaths due to breast cancer is still high. Dietary phenolics have been studied to have preventive action to inhibit cancer promotion and progression [23].

3. Plant-Derived Phenolics: Classes, Sources, and Biological Rationale

The plant-derived phenolic metabolites are generally present in fruits, vegetables, cereals, and other plant foods, and they have various biological activities and prevent various diseases, such as cancer and cardiovascular diseases [24]. Cancer is a multistage illness that is associated with excessive proliferation of cells and extended cell growth. One critical issue that has been facing clinical oncology is the need to come up with efficient agents that can regulate the growth of cancer as well as destroy the development of cancer. The anti-proliferative properties of phenolic compounds against the growth of cancer cells due to different carcinogenic agents have been studied in many research works. Plant phenolics also have antiproliferative properties because they can alter various signaling pathways that are involved in human cancers such as those in cell cycle progression and apoptosis. Particularly in this respect are the flavonoid compounds, which hold a leading position in the vegetable kingdom, and constitute one of the largest series of natural products known [25, 26]. Flavonoids constitute a big group of polyphenolic compounds that share a similar diphenyl propane skeleton and these compounds have gained interests of the scientific community due to their various physiological activities and the potential health benefits they may have. There are six major subclasses of flavonoids according to the patterns of the replacement of the basic skeleton, i.e., flavonols, flavones, flavanones, iso-flavonoids and flavan-3-ols. Quercetin, kaempferol and myricetin are flavonols that are commonly found in fruits, vegetables and plants. They have extensive anticancer activities that are mediated through various mechanisms and affect a great diversity of cancer cells. In-depth study of the anticancer properties and molecular events of various classes of flavonoids offers valuable information of biological importance and possible use in cancer prevention and treatment [27, 28].

3.1. Flavonoids and Flavonols

Herbal phenolic molecules have anti-cancer effects in a wide spectrum of cancers. A survey by Kubina *et al*. summarized all possible preclinical evidence of the antiproliferative action of phenolic compounds in cancer models and described the considerations in experimental design [29]. Flavonoids were reported to have an antiproliferative activity with efficacy observed in breast, prostate cancer models, colorectal cancer models, liver cancer models, gastric cancer models, and pancreatic cancer models. Flavonol-type compounds like quercetin and kaempferol proved the same effects with many models including the extra activity in lung cancer. Flavonoids and flavonols constitute a great family of naturally occurring plant phenolics that are abundant in fruits, herbal teas and vegetables, wine and legumes. The dietary intake of the majority of human population is the most abundant of polyphenolic compounds known as Flavonoids [30]. Dietary flavonols are associated with reduced risk of cancers of the prostate, lung, stomach, and breast. Moderate wine consumption correlates with lower incidences of lung, endometrial, esophageal, stomach, and colon cancers. Flavonols affect multiple biochemical processes during carcinogenesis. They act as immune-modulation modifiers and protect cells from free-radical damage.

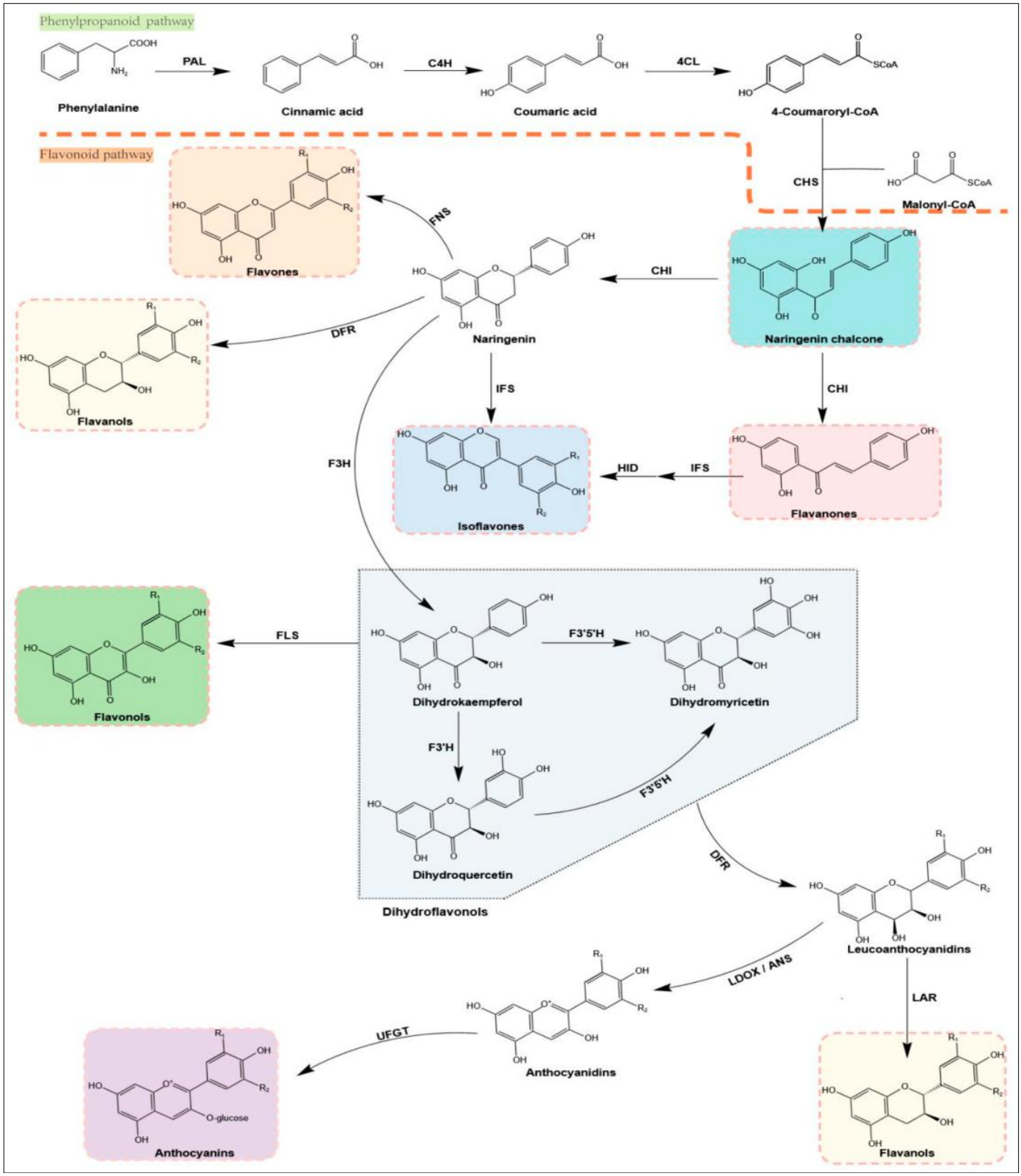

Fig 2: The pathway of flavonoid biosynthesis.

The antioxidant properties, derived from the reductive group at position 3 and the phenol group at position 5, prevent oxidative stress and promote apoptotic pathways, which inhibit cancer development. Flavonols down-regulate mutant p53, stimulate apoptosis, inhibit the cell cycle progression, and modulate signaling pathways such as MAPK and PI3K, contributing to their anticancer effect. Additionally, flavonols inhibit oxidation, detoxify xenobiotics, and exhibit anti-inflammatory actions, Figure 2 [31, 32].

3.2. Phenolic Acids and Hydroxycinnamates

Horticultural aromatics, e.g., Rosmarinus and Thymus, also benefit consumer health via antidiabetic, anti-obesity, anticancer, and antimicrobial activities. Phenolics assist metabolic syndrome prevention through antioxidant, antidiabetic, and anti-obesity features—establishing glycaemia equilibrium by inhibiting α-glucosidases (α-GluA) or dipeptidyl-peptidase IV (DPP-IV), or antagonizing adipogenesis. Chlorogenic acid (CGA, 3-caffeoylquinic) inhibits intestinal α-GluA and tumor proliferation in different cell lines [33].

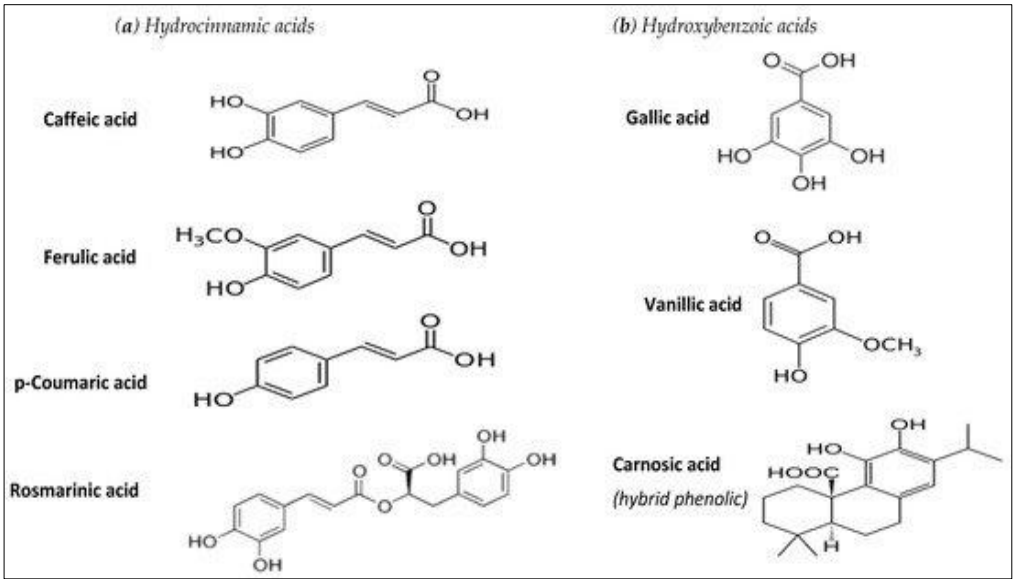

Fig 3: The chemical structure of some phenolic acids.

Hydroxycinnamate derivatives (cinnamic, coumaric, caffeic acids) elicit similar anticancer actions across several neoplasia's, including human colon cancer [34]. Using the human tumoral line SW480 or HCT116, abundance ratios between CGAs and other colonic metabolites of CGA under pre- (CGA:CGA-derived metabolites~1:15) and post-reductive (CGA:DH-CGA~1:2-1:4) models matched those expected in healthy subjects after phenolic-rich food consumption, cancer inhibiting nearly equivalently potencies for each ratio. Forcolic and para-coumaric acids at micromolar levels inhibit the growth of human colon cancer cells and transfer activity between these classes occurs in the gut. The same cell lines indicate that quercetins and chlorogenic acids, alongside colonic metabolites, prevent cancer progression at levels consistent with the human colonic environment, Figure 3 [34].

3.3. Stilbenes and Lignans

Stilbenes and lignans comprise two less common groups of phenolics, but evidence indicates that some representatives exert antiproliferative effects in cancer models. Resveratrol and several natural and synthetic derivatives have been widely investigated; many of their targets and mechanisms have been identified and linked to cell-cycle arrest and apoptosis induction [35]. Pterostilbene, a methylated analogue, also demonstrates in vitro and in vivo anticancer activity, while other stilbenes, such as sophrastilbene, viniferin, rhaponticin, and selected derivatives, show comparable but less well-characterized effects [36]. Lignans are less frequent subject of study, yet several compounds exert chemo-preventive effects by adsorbing carcinogens such as N-nitrosamines in human and murine cells. The mammalian lignans secoisolariciresinol and matairesinol, derived from dietary sources, exhibit beneficial health effects. In vitro assays indicate that both compounds inhibit a variety of cancer cell types, while syringaresinol and hydroxymatairesinol have shown promise as chemotherapeutic agents in cell studies and murine models [37].

3.4. Tannins and Polyphenolic Mixtures

Plant-derived polyphenolic compounds represent a structurally diverse class of compounds with wide-ranging biological significance. Prominent sub-classes include flavonoids (and the more specific flavonols), phenolic acids (predominantly hydroxybenzoic and hydroxycinnamic acids), stilbenes, lignans, and tannins. Numerous studies in diverse cancer models support a cytostatic or cytotoxic effect induced by diverse phenolics and yet no conclusion of a common mechanism or cellular pathway has been reached, [38] possibly due to each polyphenol acting through multiple mechanisms and highly dependent on overall cellular context. For many polyphenolic classes, such as flavonoids, phenolic acids, and stilbenes, specific structural features have been proposed to modulate activity and structure-activity relationships (SAR) have been studied, but comparable studies on tannins and polyphenolic mixtures, even individual characterization of such complex mixtures, remain rare [39]. Tannin represents a wide range of oligomeric and polymeric phenolic compounds assembled mainly through C-C or C-O bonds, and with a structure-potentiality that, combined with their much greater complexity, can be considered in a different class from other simpler phenolic compounds. Tannins—with a tendency to overlap the definition of polyphenol mixtures, especially when tannin-free standards cannot be prepared—exhibit diverse activity and anti-cancer properties in preclinical models of multiple types of cancer. Mixture of tannins and catechin-rich polyphenolic extracts from green tea, white tea, grape seeds, and wine combined with aqueous extract offers some protection from a range of mammalian tumor cell lines, examined by Alamar Blue and Crystal Violet assays. Anticancer activity of phenolic mixtures increases over the corresponding single compounds [40]. Evaluating both isolated and mixed compounds, the inhibitory effect of the mixture remains greater than any constituent; such synergistic action points to mechanisms beyond those of individual constituents [41, 42].

4. Antiproliferative Mechanisms Elicited by Phenolics in Cancer Models

Plant-derived phenolic compounds fall under 5 major classes: flavonoids, phenolic acids, stilbenes, lignans, and tannins. The compounds possess selective cytotoxicity against various cancer cell lines, such as breast, liver, and colon. Flavonoids and flavonols constitute the most widely

studied sub-classes, linked to selective and non-selective anticancer activities, modulating specific pathways associated with growth, invasion, and survival [43]. Hydroxycinnamic acids and phenolic acids exhibit selective cytotoxicity profiles that coincide with signaling pathway modulation relevant to mammary and colon cancer. Other classes, including stilbenes, lignans, polyphenols, and certain tannins, also exert antiproliferative effects, Figure 4 [43].

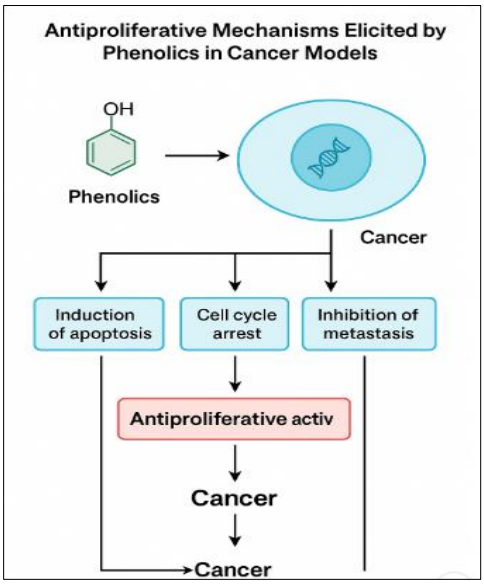

Fig 4: Mechanisms underlying the antiproliferative effects of phenolics in cancer cells.

4.1. Cell Cycle Arrest and Apoptosis Induction

Secondary metabolites can be plant-derived phenolics whose examples include flavonoids and phenolic acids, which can be found in numerous plant-based foods [44]. These are natural compounds that are commonly found throughout nature and in human diets and demonstrate a wide range of diverse biological effects including the prevention of cell proliferation, migration and invasion of different cancer cell lines [45]. Antiproliferative property of plant-derived phenolics has been associated with their capability to control the cell cycle and cause apoptosis. The apoptotic microinvolvement in the mitochondrial-pathway is aimed by the plant-derived flavonoid, 7, 47-dihydroxyflavone of Ephedra Herb [46]. Linarin from Lonicera japonica induces apoptosis by elevating intracellular $Ca2^{+}$ concentrations and regulating the expression of Bcl-2 and Bax proteins. Other research reported that cough syrup containing Salvia officinalis, Stachys officinalis, and Plantago lanceolata extracts is capable of inducing apoptosis and has potential as a complementary treatment for human cancer. Apigenin from Apium graveolens stimulates apoptosis through the mitochondrial pathway.

4.2. Modulation of Signaling Pathways

Plant-derived polyphenols exhibit a diverse range of biological activities that may help prevent the development of tumors, inhibit proliferation, induce apoptosis, and thereby protect against cancer. Experimental studies using chemically defined cancer model systems have been used to evaluate antiproliferative activity as a criterion for prioritizing natural plant compounds for evaluation as potential chemotherapeutic agents. Cell cycle arrest and caspase/tCASP activation are two mechanisms implicated in the modulation of cell growth and promotion of cell death by polyphenols. In addition to these two mechanisms, the modulation of specific signaling pathways has also been indicated as another mechanism by which phenolic compounds elicit their antiproliferative effects [47, 48].

Polyphenols from multiple, structurally distinct classes have been shown to interfere with signaling pathways that regulate cell proliferation, survival, and invasion, including the MAPK, phosphoinositide 3-kinase (PI3K)/Akt, and nuclear factor-κB (NF-κB) pathways [49]. These pathways exhibit extensive crosstalk with one another and with other relays such as the JAK/STAT pathway, providing a broad repertoire of molecular targets and mechanisms through which plant phenolics can exert antiproliferative effects that can inform structure-activity relationship studies and the design of focused libraries for exploration of anticancer phenolics, Figure 5.

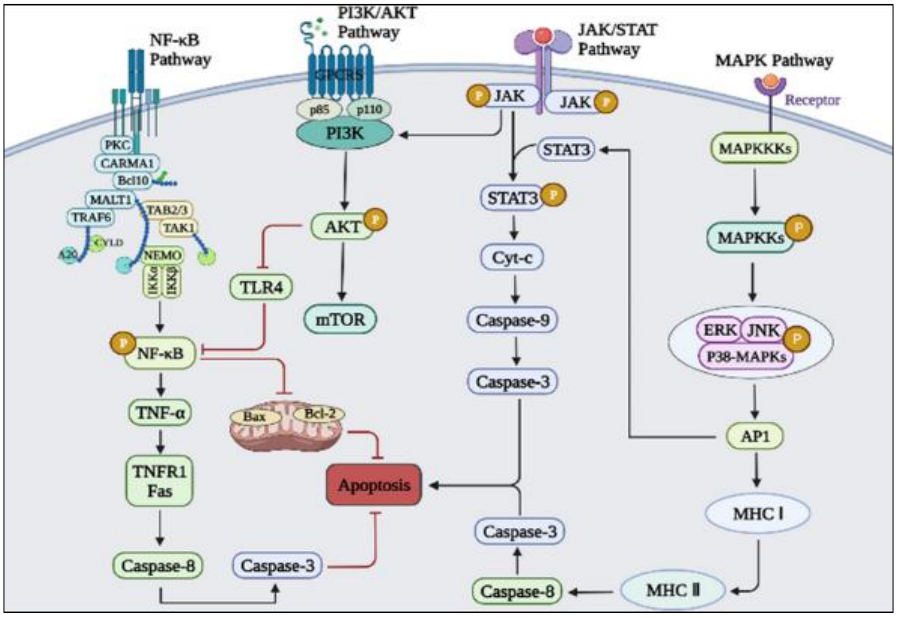

Fig 5: Antiproliferative signaling modulation induced by plant polyphenols

4.3. Epigenetic and Metabolic Effects

Dietary components have been shown to modulate epigenetic marks such as DNA methylation and histone modifications, which regulate gene expression in cancer [50]. Genistein inhibits prostate cancer cells, increasing estrogen receptor beta expression by reducing methylation of the ERbeta promoter and reversing hypermethylation of several tumor suppressor genes. Curcumin inhibits breast cancer growth through demethylation of the DLC1 tumor suppressor promoter. Resveratrol affects the methylation landscape by modifying 5-methylcytosine levels in breast cancer cells. Green tea polyphenol epigallocatechin-3-gallate causes epigenetic repression of hTERT in breast cancer cells and inhibits growth in esophageal cancer by inducing hTERT gene demethylation. Grape seed proanthocyanidins combined with trans-resveratrol decrease methylation levels of genes in human colorectal cancer and affect tumor suppressor gene expression [51]. 3, 3′-diindolylmethane alters a broad spectrum of methylated genes in prostate cancer without altering global methylation patterns. Dietary fiber influences methylation patterns in genes associated with gastric cancer and may contribute to the inverse association between dietary fiber and gastric cancer risk. Dietary factors also affect metabolites and signaling. For example, the intake of coffee has been related to a decrease in adrenaline levels, which partly prevents the development of several types of cancer. Other compounds have been reported to influence signaling. Quercetin inhibits the expression of various critical genes in cancer cells through the modulation of miRNAs involved in the regulation of Wnt signaling. Resveratrol and curcumin decrease the expression of Wnt signaling genes and proteins in different cancer cell lines. And epigallocatechin gallate downregulates VEGF and COX-2, reducing the risk of cancer [51]. Plant-derived phenolics exert an array of biological activities that link to antiproliferative actions across multiple cancer types. Flavonoids, the largest group of phenolics, target pathways governing cell cycle, apoptosis, and metabolism. Their widespread occurrence in edible plant sources provides a strong rationale for studying flavonoids as potential dietary cancer-preventive agents [51, 52]. Hydroxycinnamic acids, the most abundant group of phenolic acids in many plant-derived foods, enhance messenger RNA stability of growth-regulatory genes and convey similar protective signals in experimental models of tumoral growth. The less abundant are the stilbenes and lignans though they are found in the plant kingdom foods. The results indicating the involvement of stilbene compounds in prevention of multiple cancers and evidence of signals leading to prevention, are indicative of the worth in including this group in the evaluation of diet-induced tumorigenesis. The polyphenolic mixtures and tannins have complex interactions with cellular macromolecules, which, in theory, may result in the synergistic increase of individual components, and, hence, highlights their applicability in cancer-preventive roles [52, 53].

5. Experimental Evidence Across Cancer Models

Phenolic compounds provide a range of structures with antiproliferative activity and which occur naturally in most commonly consumed sources of food. A review of phenolic action mechanisms showed that there were six pathways of signaling that are of interest in cancer. The biological basis underlying the explanation of the anticancer effects of these compounds is based on their plant origin, which is a very strong rationale. Hypothesis about the action mechanisms stimulated by these MTT-active phenolics in cancer models have always included an early induction of either cell cycle arrest or apoptosis, possibly as a result of possible ability of these compounds to alter various signaling pathways that are essential to tumor growth, survival, and invasion [54]. Plant phenolics have a wide range of structures which give underpinning to the corresponding range of biological activities. They induce cell cycle arrest and programmed cell death in cancer models and thus captured the attention of many researchers. They are formed out of numerous food substances that are regularly consumed and are fruits, vegetables, tea, coffee, wine, and even herbs. More than 80 percent of the population consumes phenolic compounds internally on a daily basis. Epidemiological surveys have indicated high negative relationships between the consumption of plant-foods and the occurrence of different cancers and other degenerative illnesses [55]. A literature review was carried out through systematic analysis of literature to amass the evidence of MTT assays testing the anticancer effects of plant-derived phenolics against cultured cancer cells. Although phenolic activities are almost universally reported, very often the experimentally used phenolic compounds were not reported. An expanding literature records the antiproliferative properties of bioactive phenolics on breast, colorectal, liver, pancreatic, and prostate cancer and hence proposes future investigations. The classification scheme displays the great structural diversity of phenolic compounds and the need to determine the structure of different representatives to define structure-activity relationships [56].

5.1. Breast Cancer Models

Breast cancer is the most common cancer diagnosis among women and the second greatest cause of cancer-related deaths [57]. The applicability of plant-derived phenolics in this regard is that they have a broad-spectrum antiproliferative effect against the breast cancer models. Various compounds such as flavonoids (apigenin, quercetin, kaempferol, genistein, luteolin and their derivatives) and stilbesterols (resveratrol and its derivatives) have been shown to be very effective [58]. Flavonoids may act at multiple cell cycle checkpoints, with extensive investigations on quercetin and genistein. Flavonoids and other polyphenols have prompted interest in their multitargeting potential for this heterogeneous malignancy, which exhibits a considerable number of molecular and clinical signatures [58]. Estrogen-dependent and independent models have been evaluated. Flavonoids like apigenin, genistein, and resveratrol exert cytotoxicity in ER-positive lines. Both ER-positive and negative pathways remain subject to investigation, supported by evidence of inhibition in triple-negative cells. A wide range of phenolic compounds demonstrates antiproliferative effects across multiple breast cancer models [57, 58].

5.2. Colorectal Cancer Models

Phenolic compounds include a wide number of classes and subclasses of plant-derived secondary metabolites. Much evidence collected during the past four decades indicates that these phytochemicals exert inhibitory effects on the development of various types of cancer. In particular, the relevance of the antiproliferative effect of phenolic

compounds on colorectal cancer cells has been investigated. Various phenolic compounds and mixtures have been tested, although most studies have focused on only a limited number of compounds, with quercetin, resveratrol, catechins, ellagic acid, and genistein being among the most widely experimented. These compounds are generally less potent than other classes of anticancer compounds commonly used in cell proliferation assays. Nevertheless, the biochemical pathways through which these compounds exert their anticancer actions are becoming increasingly clear. Direct modulation of signaling pathways controlling cell growth, survival, and invasion is common to many phenolics applied to colon cancer cells [59]. Induction of cytostatic, cytotoxic, or both types of processes frequently accompany the addition of colon-targeted phenolic compounds to cultured cells. Typically, the strong efficacy of these compounds is directed toward proliferative cells or quiescent cells, which may explain the diminished effect of certain mixtures on more rapidly proliferating cancer cells [60].

5.3. Prostate Cancer Models

Prostate cancer (PCa), one of the most common cancers among men worldwide, exhibits twenty-fold higher geographical incidence in North America and parts of Europe. Advanced PCa is often lethal because it acquires resistance to androgen deprivation therapy. Preclinical studies provide evidence that polyphenols induce antiproliferative effects and modulate growth-regulatory signaling pathways in androgen-dependent PCa, suggesting that dietary polyphenols may delay PCa or reduce disease progression [61]. Apigenin, curcumin, and EGCG reduce cell number in the human androgen-dependent PCa cell line LNCaP. With nanocarrier delivery, EGCG potentiates antiproliferative and apoptotic effects of docetaxel against LNCaP and its variant resistant to the drug. Quercetin and resveratrol suppress LNCaP cell growth. The stilbenes resveratrol and pterostilbene inhibit cell growth. Pterostilbene additionally suppresses tumor growth in LNCaP xenografts [62]. The lignan secoisolariciresinol induces apoptosis in LNCaP and inhibits androgen-dependent LNCaP growth and transcription of androgen-responsive genes. The isoflavone genistein reduces viability in the androgen-independent PCa line PC3 and exhibits cell-cycle-regulatory effects. The isoflavonoid biochanin A suppresses tumorigenesis in the DHT-induced hamster model, underscoring PCa chemo-preventive action [63].

5.4. Liver and Pancreatic Cancer Models

Plant-derived phenolics have been reported for antiproliferative activity in several cancer types, including liver and pancreatic models. In the liver context, induction of cytotoxicity has been noted following treatment with procyanidin B2 and (+)-catechin [64]. Procyanidin B2 also induced cytotoxicity in pancreatic cells, along with a combination of quercetin and resveratrol [65].

6. Comparative Efficacy and Structure-Activity Relationships

Plant-derived phenolics have attracted attention because of their potential antiproliferative effects on cancer-cell lines [4]. To date, however, a comprehensive overview of their activity has yet to be published. An analysis of in vitro studies using the colorimetric MTT assay across diverse cancer types indicates that certain phenolics induce dose-dependent cytotoxicity. The flavylium ion, flavonols with hydroxyl substitutions at positions 3 and 5, and hydrophobic acids with a 34-styrene-type scaffold among the phenolic compounds analyzed exhibited particularly high potency [66]. The bioavailability and delivery mode must also be considered since both impact the phenolic concentrations that exert biological effects [66]. Structure-activity relationship (SAR) analysis reveals that compounds featuring the flavylium ion, hydroxylated flavonols, and 34-styrene-like hydrophobic acids are structurally similar to known potent anticancer agents and therefore worthy of further investigation. The concentration response curves produced by MTT assays are sensitive to many factors and disparities caused by variations in reporting the experimental parameters prevent accurate comparisons of the IC50 values produced by MTT. The effects of the phenolics themselves on cell viability (reduction of the MTT substrate, colorimetric interference, direct cytostatic etc.) may confound the result of observed effects. Both experimental conditions and statistical measures should be reported in a standardized way to be more reliable and comparable to the MTT-derived data. Lastly, translational opportunities and therapeutic potentiality have been considered considering the pharmacokinetics, safety, toxicology and possible off-targets of the chosen compounds [67].

6.1. Dose-Response Considerations

Plant-derived phenolic compounds evoke opposing effects on cellular interactions, inclusive of cellular proliferation and cell viability. MTT-based cytotoxicity evaluations post exposure to plant-derived phenolics have been routinely documented in breast, prostate, liver, colorectal, and other cancer models [67]. The MTT biochemical assay is an extensively employed cytotoxicity determination procedure for biological research and drug screening. Induced cell death or cessation to proliferate forms a fundamental aspect of potential anticancer agents. The MTT assay quantifies these events through conversion of yellow tetrazolium salt MTT into purple formazan in viable cells. The concentrations of antihyperglycemic and hypolipidemic plant-derived phenolics, such as gallic acid, quercetin, ellagic acid, catechol, and epicatechin designated viable cell count percentages and viability enhancement percentage post-exposure and adherent to Connolly *et al*.'s evaluation framework. Antiproliferative properties of these organotin against BRL-3A, BHK-21, and other cells were examined through MTT-based cytotoxicity assays [68].

6.2. Influence of Bioavailability and Delivery

A crucial factor in the therapeutic activity of chemo-preventive agents is their bioavailability, which affects the total delivered dose and tissue distribution. Formulations can influence the absorption and distribution of various compounds, including resveratrol, curcumin, and soy isoflavones. Guidelines exist for the generation of phytochemical delivery formulations, or systems, which aim to increase stability, absorption, or targeting of specific tissues [58]. Such methodologies have been reported to enhance the antiangiogenic and antitumor activity of these agents. Multiple lipid-based delivery systems, currently employed for phytochemicals, aim to increase water solubility and stability, prevent rapid clearance, reduce

intestinal and hepatic metabolism, improve transport to circulation, or facilitate targeting of cancer cells [69].

6.3. Structural Features Correlating with Activity

Global cancer incidence is projected to rise by 47% from 2008 to 2030. Current anticancer therapies have undesirable side effects and are often not curative. New, safe, and effective agents are therefore urgently needed to treat and prevent cancer. Phytochemicals are naturally occurring bioactive compounds in plants that may prevent and treat cancer with few side effects. Growing interest has emerged in phenolic compounds, which are widely distributed in plants and possess considerable antioxidant activity [70]. The minuscule polyphenol-containing plant Terminalia benzo affects cellular proliferation in human hepatocellular carcinoma HepG2 cells through apoptotic and cell cycle arrest mechanisms. Extracts inhibited the growth of all cancer cell lines tested, but not non-cancerous epithelial, fibroblast, keratinocyte, or skin cells [4]. Polyphenolics also prevent chemically induced experimental tumors, inhibit the proliferation of diverse cultured tumor cells, and block the growth of solid tumors in rodent models. These compounds are active against models of breast carcinoma, colorectal carcinoma, and prostate cancer, making them strong candidates for further study. The anticancer potential of T. benzo against HepG2 cells and pancreatic cancer cells remains unexplored, as does the impact of various solvents on efficacy [71].

7. Methodological Variability and Data Interpretation

Cytotoxicity is commonly assessed in cancer research to characterize the effect of phenolic compounds on cell lines [71]. Common cytotoxicity assays use tetrazolium salts such as thiazolyl blue tetrazolium bromide (MTT), resazurin (Alamar blue), and the cell counting kit-8 (CCK-8) to provide an indirect readout of the loss of cell viability. Coupled with proposed antiproliferative mechanisms, reported IC50 values across multiple cancer cell lines provide a wealth of information on the anticancer potential of plant-derived phenolics [72].

7.1. Assay Interference by Phenolics

Phenolic compounds may also interfere with the MTT assay causing considerable inaccurate estimations of antiproliferative activity. A number of phenolics have been reported to directly reduce MTT to formazan including ellagitannins and flavonoids [73]. Further coloration can be obtained in situations where the treating solvent reacts with MTT or formazan. Furthermore, a number of plant-phenolics have absorbances in the visible light and some are auto-fluent. Therefore, MTT responses measurements around the wavelength of the light-source should be avoided. Interference can also be reduced by adding the MTT at defined times since the plant phenolics can also alter the color or the opaqueness of the first few hours of exposure. The concomitant determination of treatment effects on cell growth is complicated by the possibility of cytostatic behavior: the determination of IC50 values on data sets where this occurs should be done with caution. On the other hand, when the relative change on the adherent-cell density is reported but not the total cell concentration, results obtained at overlapping time windows are likely to give false potencies.

7.2. Reproducibility and Cross-Study Comparisons

Dot-to-dot cytotoxicity measures of MTT obtained by cross-study comparisons deviate widely within and between cancer settings. There is a minimum of three orders of magnitude of the reported IC50 values of individual phenolics, and overall phenolic concentrations often differ by two to three-fold at a particular IC50 estimate. As a supplement to these differences, the qualitative division of the treatment results, which is differentiated into cytotoxic and cytostatic, is not consistent in terms of terms and meaning. Percentages of absolute growth-inhibition at IC_{50}, and IC_{20} etc. Benchmarks are also unstandardized. High-throughput screening of plant-derived phenolics in overlapping cancer models, canonical compound libraries, and canonical viability measurements and therefore allows the better assessment of factors contributing to reported efficacy [74, 75].

8. Methodological Improvements for MTT-Based Assessments

The MTT-based assay requires the evaluation of plant-derived phenolics which are difficult to evaluate due to physiological interactions that may confound the interpretation. All assays that rely on the reduction of tetrazolium salts to colored formazan products, including MTT and alternatives such as XTT, suffer from this problem when applied to substances that interfere with the marker to yield false-positive results misrepresenting the actual population of living cells [74]. Moreover, adjustment of bioavailability or exposure time based on indications of assumed biochemical modes of action may more closely match expected mechanisms of action but may inadvertently produce positive artifacts when bioactive metabolites themselves exert additional effect. Users must determine a compromise that balances target prospects against the risk of misleading results propagated through the literature if an intermediate condition nevertheless leads to diminished activity [75, 76]. Although the MTT assay is widely used and considered sufficiently robust for estimation of time-dependent cytotoxicity in anticancer evaluations, complete experimental design specifications are rarely provided, complicating appraisal and cross-study comparisons. Despite the fact that core conditions were maintained, it has been observed in studies that reported MTT-derived IC_{50} estimates of the same substance a large amount of variation not only in concentrations of test agents used, but also in dishes and solvents used [77, 78].

9.3. Recommendations for Integrated Evaluation

Assessment of plant-derived phenolics by the MTT array is typical of a developing field. The studies using MTT determine these compounds in varying cancer models with different endpoints, treatment regimens and dose-response behaviors. Conditions of the experiment are not standardized, and reporting of data is not consistent. The variability leads to issues with interpretation, but the proponents of the plant-derived phenolics point out that there is overall evidence that their hypothesis is correct, a MTT-based test should be an option to make as long as a solid preliminary screening and judicious experimental design. Since MTT data are commonly highlighted in the spotlight, it is advisable to apply integrated evaluation frameworks in order to synthesize data comprehensively, interpret it, and gain a mechanistic understanding. Another

aspect of variety in the field of MTT-based evaluation is represented by different cancer models. Complete frameworks of combining MTT data and non-MTT end-points or omics datasets of other dimensions of evaluation would make studies more comparable, clarify mechanistic details, and help locate appropriate delivery mechanisms and co-formulants. Additional methodologies would expand the knowledge of the structure-activity interaction and form the basis of developing a more definite biopharmaceutical map, thereby facilitating more preclinical development and clinical translation.

10. Conclusion

Various plant-based phenolics have the important antiproliferative properties on representative cancer models with concentration levels that are realistic about the levels of phenolics that can be acquired in the diet. Relevant cancer phenolic assessments prefer MTT assessments, but possess pronounced methodological heterogeneity and confounding outcomes. Solving these problems will enable the promotion of the mechanistic understanding and the therapeutic potential of bioactive plant-derived compounds on cancer control.

Funding

There is no funding

Declaration of Competing Interest

The authors say they don't have any known personal or financial relationships or financial interests that could have seemed to affect the work in this study.

Acknowledgment

This review article is supported by the College of Veterinary, University of Basrah, Iraq.

References

1. Carocho M, Ferreira ICF. The role of phenolic compounds in the fight against cancer - a review. 2013.
2. Dehelean CA, Marcovici I, Soica C, Mioc M. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules. 2021;26(21).
3. Shukla S, Shukla AK, Upadhyay AM, Ray N, Fahad FI. Molecular insight and antioxidative therapeutic potentials of plant-derived compounds in breast cancer treatment. Onco. 2025.
4. Rummun N, Rondeau P, Bourdon E, Pires E, *et al*. *Terminalia bentzoë*, a Mascarene endemic plant, inhibits human hepatocellular carcinoma cell growth in vitro via G0/G1 phase cell cycle arrest. 2020.
5. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. 2021.
6. Li T, Wang S, Ling Q, Sun J, Yang N, Fu Z. Cell viability, drug screening, and mechanism study under mild phototherapy integrated chemotherapy (PIC). Adv Mater. 2025.
7. Job V, Bonil L, Coupeau D, Penninckx S, *et al*. Development of high-throughput screening viral titration assay: proof of concept through two surrogate viruses of human pathogens. Biology Methods. 2025.
8. Ghasemi M, Turnbull T, Sebastian S. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J. 2021.
9. Buranaamnuay K. The MTT assay application to measure the viability of spermatozoa: a variety of the assay protocols. Open Vet J. 2021.
10. Soltani A, Shams Abadi MS, Raeisi M, *et al*. Apoptosis-inducing plant-based phenolic compounds are effective on leukemia cell lines. Curr. 2023.
11. Wani AK, Akhtar N, Mir TG, Singh R, Jha PK, Mallik SK. Targeting apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. Biomolecules. 2023.
12. Kinjo J, Nakano D, Fujioka T, Okabe H. Screening of promising chemotherapeutic candidates from plant extracts. 2016.
13. Tabassam Q, Ahmed S, Mehmood T. Phytochemical investigation and effective therapeutic potential of plant extracts against breast and ovarian cancer cell lines: compounds from *Zizyphus mauritiana*. Cell Mol Biol. 2021;67(1):1-10.
14. Asuzu PC, Trompeter NS, Cooper CR, Besong SA. Cell culture-based assessment of toxicity and therapeutics of phytochemical antioxidants. Molecules. 2022.
15. Barboza JR, Pereira FAN. Molecular mechanisms of action and chemosensitization of tumor cells in ovarian cancer by phytochemicals: a narrative review on pre-clinical and clinical studies. Phytother Res. 2023.
16. Shahi S, Singh SK. Phytochemical analysis and characterization of cancer-cure medicinal plants: a comprehensive review. Int J Environ Sci. 2025.
17. Akter S, Addepalli R, Netzel ME, Tinggi U, *et al*. Antioxidant-rich extracts of *Terminalia ferdinandiana* interfere with estimation of cell viability. 2019.
18. Vidal-Jove J, Serres X, Vlaisavljevich E, *et al*. First-in-man histotripsy of hepatic tumors: the THERESA trial, a feasibility study. Int J. 2022;XX(YY):ZZ-ZZ.
19. Elsacker E, Zhang M. Fungal engineered living materials: the viability of pure mycelium materials with self-healing functionalities. Adv Funct Mater. 2023.
20. Schurr M, Graf J, Junne F, Giel KE. Psychotherapy in patients with long/post-COVID: a systematic review on the feasibility, acceptability, safety, and efficacy of available and emerging interventions. J Psychosom Res. 2025.
21. Zheng Y, Li P, Shen J, Yang K, Wu X, Wang Y. Comprehensive comparison of different parts of *Paeonia ostii*, a food-medicine plant, based on untargeted metabolomics, quantitative analysis, and bioactivity. Front Plant Sci. 2023.
22. Pissard A, Baeten V, Arnould Q, Rogez H, *et al*. Characterization and discrimination of pure standards of phenolic compounds using FTIR spectroscopy in the terahertz range. Foods. 2025.
23. Rajput A, Osmani RAM, Singh E, Banerjee R. Cancer: a sui generis threat and its global impact. In: Advanced Cancer. Elsevier; 2022.
24. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. 2010.
25. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants—natural health products for human health. Molecules. 2023.
26. Shrihastini V, Muthuramalingam P, Adarshan S. Plant-derived bioactive compounds, their anti-cancer effects

and in silico approaches as an alternative target treatment strategy for breast cancer. Cancers. 2021.
27. Faisal A, Abbas MA, Mohsein OA. Histological and cytokine-based biomarkers in the early diagnosis of cancer. Indones J Health Sci Med. 2025;2(3):10-21070.
28. Ganai SA, Sheikh FA, Baba ZA, Mir MA, *et al*. Anticancer activity of the plant flavonoid luteolin against preclinical models of various cancers and insights on different signalling mechanisms modulated. Phytother Res. 2021.
29. Kubina R, Iriti M, Kabała-Dzik A. Anticancer potential of selected flavonols: fisetin, kaempferol, and quercetin on head and neck cancers. 2021.
30. Chahar MK, Sharma N, Dobhal MP, Joshi YC. Flavonoids: a versatile source of anticancer drugs. 2011.
31. Oczkowski M, Dziendzikowska K. Dietary factors and prostate cancer development, progression, and reduction. Nutrients. 2021.
32. Galván-Portillo M, Vázquez-Salas RA. Dietary flavonoid patterns and prostate cancer: evidence from a Mexican population-based case-control study. J Nutr. 2022.
33. Cattivelli A, Conte A, Tagliazucchi D. Quercetins, chlorogenic acids and their colon metabolites inhibit colon cancer cell proliferation at physiologically relevant concentrations. 2023.
34. Hussein AD, Dahham AH, *et al*. Emerging biomarkers in cancer detection and prognosis: a comprehensive review. Int J Immunol. 2025;7(1):1-12.
35. Hazafa A, Iqbal MO, Javaid U, Tareen MBK. Inhibitory effect of polyphenols (phenolic acids, lignans, and stilbenes) on cancer by regulating signal transduction pathways: a review. Transl Oncol. 2022;15(10):101-112.
36. González-Sarrías A, Espín-Aguilar JC. Main determinants affecting the antiproliferative activity of stilbenes and their gut microbiota metabolites in colon cancer cells: a structure-activity relationship. Int J. 2022.
37. Takashima H, Tagami T, Kato S, Pae H, Ozeki T. Printing of an apigenin-loaded mucoadhesive film for tailored therapy to oral leukoplakia and the chemopreventive effect on a rat model of oral carcinogenesis. Pharmaceutics. 2022.
38. Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M. Anticancer efficacy of polyphenols and their combinations. 2016.
39. Naif NH, Hassan Z, *et al*. The impact of inflammatory and adipokine biomarkers on breast cancer progression and patient outcomes. Bull Pharm Sci Assiut Univ. 2025;48(1):511-522.
40. Sharifi-Rad J, Seidel V, Izabela M. Phenolic compounds as Nrf2 inhibitors: potential applications in cancer therapy. Cell Commun. 2023.
41. Matulja D, Vranješević F, Kolympadi Markovic M, *et al*. Anticancer activities of marine-derived phenolic compounds and their derivatives. Molecules. 2022.
42. Melo Ferreira D, Oliveira MBPP, Alves RC. A comprehensive review of the antitumor activity of olive compounds: the case of olive oil, pomace, and leaf extracts, phenolic alcohols, secoiridoids. Antioxidants. 2025.
43. Farhan M, Rizvi A. Understanding the prooxidant action of plant polyphenols in the cellular microenvironment of malignant cells: role of copper and therapeutic implications. 2022.
44. Dhupal M, Chowdhury D. Phytochemical-based nanomedicine for advanced cancer theranostics: perspectives on clinical trials to clinical use. 2020.
45. Chaudhry GS, Akim AM, Sung YY, Tengku Sifzizul TM. Cancer and apoptosis: the apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. 2022.
46. Ghazi MH, *et al*. Evaluation of association between tumor markers, hormonal receptors, inflammatory biomarkers and breast cancer. Arch Breast Cancer. 2026;13(1).
47. Niu C, Zhang J, Okolo PI 3rd. Liver cancer wars: plant-derived polyphenols strike back. Med Oncol. 2024.
48. Lyubitelev A, Studitsky V. Inhibition of cancer development by natural plant polyphenols: molecular mechanisms. Int J Mol Sci. 2023.
49. Mohammed Z, *et al*. The role of herbal extracts in modulating inflammatory cytokines and cancer biomarkers. Egypt J Med Microbiol. 2026;35(1).
50. Carlos-Reyes Á, López-González JS, Meneses-Flores M, Gallardo-Rincón D, *et al*. Dietary compounds as epigenetic modulating agents in cancer. 2019.
51. Jasek K, Kubatka P, Samec M, Liskova A, *et al*. DNA methylation status in cancer disease: modulations by plant-derived natural compounds and dietary interventions. 2019.
52. Debnath P, Deb VK, Nath S, Guha A. Structure-activity relationship of plant-derived bioactive compounds in anticancer therapy. In: Plant Derived Bioactive. 2025.
53. Muhammed TM, *et al*. Comparative analysis of PSMA, PSA3, and TMPRSS2-ERG as diagnostic and prognostic biomarkers in prostate cancer patients. Int J Endocrinol (Ukraine). 2025;21(7):728-734.
54. Cid-Gallegos MS, Sánchez-Chino XM. Anticarcinogenic activity of phenolic compounds from sprouted legumes. Food Rev. 2022.
55. Benarba B, Belhouala K, Pandiella A. Plant and spice mixtures as promising anticancer agents: in silico drug design predictions targeting cancer cell proliferation through cell cycle arrest. J Comput. 2025.
56. da Silva GB, Rocha KG, Bagatini MD, Kempka AP. Anticancer properties of phenolic acids and cell death signaling pathways: a 20-year bibliometric analysis (2003-2023). Food Biosci. 2025.
57. Sakban GH, *et al*. Exploring biomarkers, adipokines, and cytokines in patients with colon cancer: a comparative study with healthy controls. Regul Mech Biosyst. 2025;16(3):e25150.
58. Mocanu MM, Nagy P, Szöllősi J. Chemoprevention of breast cancer by dietary polyphenols. 2015.
59. Bakrim S, El Omari N, El Hachlafi N, Bakri Y, Lee LH. Dietary phenolic compounds as anticancer natural drugs: recent update on molecular mechanisms and clinical trials. Foods. 2022;11(5).
60. Rahman MM, Rahaman MS, Islam MR, Rahman F, *et al*. Role of phenolic compounds in human disease: current knowledge and future prospects. Molecules. 2021.

61. Costea T, Nagy P, Ganea C, Szöllősi J, *et al*. Molecular mechanisms and bioavailability of polyphenols in prostate cancer. 2019.
62. Ahmad N, Qamar M, Yuan Y, Nazir Y, Wilairatana P, *et al*. Dietary polyphenols: extraction, identification, bioavailability, and role for prevention and treatment of colorectal and prostate cancers. Molecules. 2022;27(12).
63. Ghazi MH, *et al*. Early detection of prostate cancer using novel ELISA-based biomarkers: insights into inflammatory and tumor-specific pathways. Asian Pac J Cancer Prev. 2025;26(10):3833-3839.
64. Ekingen Genc D, Ozbek O, Oral B, Yıldırım R, *et al*. Phytochemicals in pancreatic cancer treatment: a machine learning study. 2023.
65. Mesas C, Quiñonero F, Doello K, Revueltas JL, *et al*. Active biomolecules from vegetable extracts with antitumoral activity against pancreas cancer: a systematic review (2011-2021). 2022.
66. Kalinowska M, Świsłocka R, Wołejko E. Structural characterization and evaluation of antimicrobial and cytotoxic activity of six plant phenolic acids. PLoS One. 2024.
67. Yüce H, Şahin Y, Türkmen NB, Özek DA. Apoptotic, cytotoxic and antimigratory activities of phenolic compounds. J Evol Biol. 2022.
68. Kumar A, Rai Y, Bhatt AN. Anti-cancer drug-mediated increase in mitochondrial mass limits the application of metabolic viability-based MTT assay in cytotoxicity screening. Cytotechnology. 2024.
69. Mouhid L, Corzo-Martínez M, Torres C, Vázquez L, *et al*. Improving in vivo efficacy of bioactive molecules: an overview of potentially antitumor phytochemicals and currently available lipid-based delivery systems. 2017.
70. Soerjomataram I, Bray F. Planning for tomorrow: global cancer incidence and the role of prevention 2020-2070. Nat Rev Clin Oncol. 2021.
71. Wu J, Xiao F, Zheng Y, Lin Y, *et al*. Worldwide trend in human papillomavirus-attributable cancer incidence rates between 1990 and 2012 and Bayesian projection to 2030. Cancer. 2021.
72. Lopez-Corona AV, Valencia-Espinosa I, *et al*. Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (*Rubus idaeus*): a general review. Antioxidants. 2022.
73. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. 2010.
74. Kumar N, Afjei R, Massoud TF, Paulmurugan R. Comparison of cell-based assays to quantify treatment effects of anticancer drugs identifies a new application for Bodipy-L-cystine to measure apoptosis. 2018.
75. Gasparini LS, Macedo ND, Pimentel EF, Fronza M, *et al*. In vitro cell viability by CellProfiler® software as equivalent to MTT assay. 2017.
76. Nadar AP, Sellappan K. Production of *Syzygium laetum*-engineered ZnO nanoparticles: from physicochemical properties to in vitro bioactivities and in vivo ecotoxicity assessment. Biomed Mater Devices. 2025.
77. Badawy HAE, Osman A, Ahmed TAE, *et al*. Evaluation of plant-derived biomaterials for the development of tissue-engineered corneal substitutes. J Biomed. 2024.
78. Pazin WM, Miranda RR, Toledo KA, Kjeldsen F. pH-dependence cytotoxicity evaluation of artepillin C against tumor cells. Life. 2023.