Review Article

Antitumor Phenylpropanoids Found in Essential Oils

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Received 5 July 2014; Accepted 12 October 2014

Academic Editor: Gagan Deep

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The search for new bioactive substances with anticancer activity and the understanding of their mechanisms of action are high-priorities in the research effort toward more effective treatments for cancer. The phenylpropanoids are natural products found in many aromatic and medicinal plants, food, and essential oils. They exhibit various pharmacological activities and have applications in the pharmaceutical industry. In this review, the anticancer potential of 17 phenylpropanoids and derivatives from essential oils is discussed. Chemical structures, experimental report, and mechanisms of action of bioactive substances are presented.

1. Introduction

Cancer is a global health concern that causes mortality in both children and adults. More than 100 distinct types and subtypes of cancer can be found within specific organs [1]. Despite the success of several cancer therapies, an ideal anticancer drug has not been discovered, and numerous side effects limit treatment. However, research into new drugs has revealed a variety of new chemical structures and potent biological activities that are of interest in the context of cancer treatment.

Essential oils are natural products that are a mixture of volatile lipophilic substances. The chemical composition of essential oils includes monoterpenes, sesquiterpenes, and phenylpropanoids, which are usually oxidized in an aliphatic chain or aromatic ring. Several studies have shown that this chemical class has several biological activities, including analgesic, anticonvulsant, and anti-inflammatory effects [2–4]. Antitumor activity has been reported for essential oils against several tumor cell lines [5–7], and these oils contain a high percentage of phenylpropanoids, which are believed to contribute to their pharmacological activity [8, 9].

This paper presents a literature review of phenylpropanoids from essential oils with respect to antitumor activity, with chemical structures and names of bioactive compounds provided. The phenylpropanoids presented in this review were selected on the basis of effects shown in specific experimental models for evaluation of antitumor activity and/or by complementary studies aimed at elucidating mechanisms of action (Table 1). The selection of essential oil constituents in the database was related to various terms, including essential oils and phenylpropanoids, as well as names of representative compounds of chemical groups, and refined with respect to antitumor activity, cytotoxic activity, and cytotoxicity. The search was performed using scientific literature databases and Chemical Abstracts Service (CAS) in November 2013.

2. Phenylpropanoids

2.1. Eugenol. Eugenol is the active component of essential oil isolated from clove (Syzygium aromaticum) and has antimutagenic, antigenotoxic, and anti-inflammatory properties [10]. Eugenol also has cytotoxic activity. This drug can induce cell death in several tumor and cell types: mast cells [11–13], breast adenocarcinoma [13], melanoma cells [14–16], leukemia [14, 17], colon carcinoma [18], cervical carcinoma...
plasia, epidermal ODC activity, protein expression of iNOS and COX-2, and secretion of proinflammatory cytokines, all of which are classical markers of inflammation and tumor promotion [42]. In addition, eugenol has been shown to produce antioxidant effects via free radical scavenging activity and reduction of ROS [22, 36, 43]. Atsumi and collaborators [36] showed that visible-light irradiation and elevation of the pH of the eugenol-containing medium resulted in significantly lower cell survival in HSG cultures in comparison with eugenol alone.

In vivo murine assays have also demonstrated the antitumor potential of eugenol. Treatment of female B6D2F1 mice bearing B16 melanoma allografts with 125 mg/kg of eugenol resulted in a small, but highly significant (P = 0.0057), 24-day tumor growth delay. Furthermore, the treated animals had no fatalities that were attributed to metastasis or tumor invasion, which is indicative of the ability of eugenol to suppress melanoma metastasis [15]. Jaganathan and collaborators [40] demonstrated that eugenol stimulated production of melanin formation by more than 42% in the B16 melanoma cell line in vitro, with cytotoxicity in 5% of cells. At a higher concentration of 200 µg/mL 23% cytotoxicity was observed, which demonstrated that eugenol could be useful as a skin-whitening agent for the treatment of hyperpigmentation [45].

Furthermore, it has been demonstrated that eugenol, when mixed with zinc oxide, has a restorative effect on dental erosion and demineralization [46]. Using human dental pulp cells (D824) it was observed that eugenol had a cytotoxic effect, with reduction of cell growth and inhibition of colony-forming cell [35]. D824 cells have the potential for metabolic activation, because they are a mixed-cell population composed of many types of cells, and thus the cytotoxic activity of eugenol could be attributable to eugenol metabolites. However, Maysa and collaborators [46] showed a hemolytic effect of eugenol, which could be a possible side effect of this drug. In addition, Anpo and collaborators [35] showed that eugenol reduced growth and survival of human dental pulp cells, as well as collagen synthesis and bone sialoprotein (BSP) expression, which play a critical role in physiological and reparative dentinogenesis. Eugenol is a phenylpropanoid with promising antitumor drug profile. Further studies to elucidate the mechanisms that mediate the adverse effects of eugenol are necessary.

2.2. Methyleugenol, Isoeugenol, Methylisoeugenol, and 1’-Hydroxymethyleugenol. Methyleugenol is a substituted alkylbenzene found in a variety of foods and essential oils. It is structurally similar to eugenol and found in many plant species [47]. Methyleugenol produced cytotoxic effects in rat and mouse hepatocytes [47, 48] and leukemia [48]. Methyleugenol also produced genotoxicity in mice [47] and in cultured cells [49] and caused neoplastic lesions in the livers of Fischer 344 rats and B6C3F1 mice [47].

Isoeugenol is a phenylpropanoid produced by plants. As a flavoring agent, isoeugenol is added to nonalcoholic drinks, baked foods, and chewing gums. In male F344/N rats,
Table 1: Essential oil phenylpropanoids with antitumoral activity.

| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|---------------------------------------|-------------------------|-----------|
| Anaphylaxis model | Apoptotic manifestations via phospho-ser 15-p53 into mitochondria | Mast cells | [11] |
| Skin carcinogenesis model | Inhibition of the proliferation associated genes c-Myc and H-ras and antiapoptotic gene Bcl2 along with upregulation of proapoptotic genes Bax, p53, and active caspase-3 | Mice | [12] |
| Trypan-blue assays | Cytotoxic activity | B16-F10, Sbc12, WM3211, WM98-1 and WM1205Lu, PC-3, human gingival fibroblasts, oral mucosal, neutrophils—male guinea pig, rat hepatocytes cells Sbc12, WM3211, WM98-1, and WM1205Lu cells | [14, 15, 23, 32, 33, 48, 49] |
| Melanoma cell proliferation | Deregulation of the E2F family of transcription factors, transcriptional activity of E2F1 | P-815, K-562, CEM, and MCF-7 cells | [15] |
| Flow cytometry analysis | Cytotoxic activity | B16-F10, P-815, K-562, CEM, MCF-7, MCF-7 gem, HeLa, DU-145, KB, HSG, human dental pulp, murine peritoneal macrophages HL-60, HepG-2, 2B16, cells | [13, 19–22, 25–29, 38, 45, 46, 48] |
| VL irradiation time | Antioxidative reactivity | Caco-2 cells and VH10 fibroblasts | [18] |
| MTT assay | Cytotoxic activity | HeLa cells | [19] |
| DPPH assay | Antioxidative activity | Human erythrocytes | [20] |
| Flow cytometer analysis | Enhanced the accumulation of cells in the S and G2/M phase which may be unable to divide | | |
| DAPI staining | Increase in the number of apoptotic cells | | |
| In vitro hemolytic activity | Hemolytic activity | | |
| Caspase-3 colorimetric assay | Induce caspase 3-mediated apoptosis | | |
| RT-PCR | Anticancer activities via apoptosis induction and anti-inflammatory downregulation of Bcl-2, COX-2, and IL-1β | | |
| RT-PCR | Downregulated the expression of Bcl-2, COX-2, and IL-1β | HeLa cells | [20] |
| Flow cytometer analysis | Increased population of cells G2/M phase by 4.5-fold | PC-3 cells | [24] |
| Western blot and RT-PCR analysis | Reduced expression of antiapoptotic protein Bcl-2 and enhanced expression of proapoptotic protein Bax | | |
| DPPH radical-scavenging activity | Formation of dimers | HSG cells | [25] |
| ELISA | Reduced the nicotine-induced ROS, NO generation, and iNOSII expression | Murine peritoneal macrophages | [27] |
| Compound                                      | Experimental protocol                                      | Antitumoral activity and/or mechanism                                                                 | Animal/cell line tested            | Reference |
|-----------------------------------------------|------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------------------------------|-----------|
| Spectrophotometric analysis                   | Increase in LDH release                                    |                                                                          | DU-145 and KB cells               | [28]      |
| ESR analysis                                  | Activity of the production of phenoxy radicals with most efficiently scavenged reactive oxygen |                                                                          |                                   |           |
| Laser cytometry analysis                      | Production of ROS induced by VL-irradiated is significantly affected by pH |                                                                          | HSG cells                         | [29]      |
| Antioxidants production                       | Produced antioxidants in alkaline solutions                |                                                                          | Human salivary gland and oral squamous cells | [30]      |
| DPPH assay                                    | Apoptosis-inducing effect                                  |                                                                          | HGF and HSG cells                 | [31]      |
| TBA analysis lipid oxidation                  | Decreased cellular ATP level in a concentration- and time-dependent manner |                                                                          | Oral mucosal fibroblasts           | [32]      |
| ATP assay                                     | Intracellular glutathione levels                           |                                                                          | HFF and HepG2 cells               | [33]      |
| NR assay                                      | Reduction in the intracellular level of GSH                |                                                                          | HSG cells                         | [34]      |
| Dichlorofluorescein assay                     | Induced a dose-dependent increase of aberrant cells       |                                                                          | V79 cells                         | [35]      |
| CAs assay                                     | Inhibition of topoisomerase II                             |                                                                          | Swiss mice                        | [36]      |
| Croton oil induced skin carcinogenesis        | Inhibition of the proliferation associated genes c-Myc and H-ras and antiapoptotic gene Bcl2 along with upregulation of proapoptotic genes Bax, p53, and active caspase-3 | Declined of hyperplasia, epidermal ODC activity, and protein expression of iNOS, COX-2, and secretion of proinflammatory cytokines | Swiss mice | [37]      |
| DMBA/TPA-induced carcinogenesis in murine skin | Upregulation of p53 expression with a concomitant increase in p21WAF1 levels in epidermal cells indicating induction of damage to the DNA |                                                                          |                                   |           |
| TUNEL assay                                   | Induces apoptosis in melanoma tumors                       |                                                                          | WM1205Lu cells                    | [24]      |
| Flow cytometric analysis                      | cDNA array analysis showed that eugenol caused deregulation of the E2F family of transcription factors |                                                                          | WM1205Lu cells                    | [38]      |
| TUNEL assay                                   | Induces apoptosis in melanoma tumors                       |                                                                          | HL-60 and HepG-2 cells            | [39]      |
| DPPH assay                                    | Antioxidative properties                                   |                                                                          | SK-OV-3, XF-498, and HCT-15 cells | [40]      |
| Sulforhodamine B assay                        | Cytotoxic activity                                          |                                                                          |                                   |           |
| Marine Ehrlich ascites and solid carcinoma models | Inhibit the growth of Ehrlich ascites                      |                                                                          | BALB/c mice                       | [41]      |
| DPPH assay                                    | Antioxidation activity                                     |                                                                          | HepG2 cells                       | [42]      |
| Western blot analysis                         | Decreased the protein expression of BSP in a concentration-dependent manner |                                                                          | Human dental pulp cells           | [43]      |
| DPPH assay                                    | Antioxidant effect                                         |                                                                          | Raw 264.7 cells                   | [44]      |
| VL irradiation/MTT assay                     | Generation of eugenol radicals                             |                                                                          | HSG and HGF cells                 | [35]      |
| Laser cytometry                               | Generation of ROS                                          |                                                                          |                                   |           |
| ESR analysis                                  | Produced phenoxy radicals                                  |                                                                          | HSG and HGF cells                 | [36]      |
| Superoxide generation/spectrophotometer       | Stimulation the production of superoxide (O$_2^-$)         |                                                                          | Neutrophils—male guinea pig       | [37]      |
| Compound                                    | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested                                      | Reference |
|---------------------------------------------|------------------------|---------------------------------------|-------------------------------------------------------------|-----------|
| Methyleugenol (4-allyl-1,2-dimethoxybenzene) | DPPH assay             | Antioxidative properties               | HL-60 and HepG-2 cells                                      | [48]      |
|                                             | UDS assay               | Cytotoxicity and genotoxicity effects  | B6C3F1 mouse hepatocytes                                     | [47]      |
|                                             | L-Lactate assay         | Cytotoxic effect                       | F-344 rat hepatocytes                                        |           |
|                                             | MTT assay               | Cytotoxic activity                     | SK-Mel-28, LCP-Mel, LCM-Mel, PNP-Mel, CN-MelA, and GR-Mel cells | [16, 48] |
|                                             | DPPH assay              | Antioxidative properties               |                                                            |           |
|                                             | WST assay               | Cytotoxic and genotoxic properties     | V79 cells                                                   | [49]      |
|                                             | SRB assay               |                                       |                                                            |           |
|                                             | Corn oil gavage         | Carcinogenic activity is based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) | F344/N rats and B6C3F1 mice | [50] |
|                                             | Trypan-blue exclusion assay | Cytotoxic activity                   | Rat hepatocytes                                             | [55]      |
| Isoeugenol (4-propenyl-2-methoxyphenol)     | MTT assay               | Cytotoxic activity                     | HSG cells                                                   | [29]      |
|                                             | DPPH radical-scavenging activity | Cytotoxic activity                   |                                                            |           |
|                                             | Dichlorofluorescein assay | Cytotoxic activity and/or mechanism    |                                                            |           |
|                                             |                        |                                       |                                                            |           |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|-------------------------|-----------|
| Methyl-isoeugenol (4-allyl-1,2-dimethoxybenzene) | MTT assay | Inhibition of cell proliferation | WM266-4, SK-Mel-28, LCP-Mel, LCM-Mel, PNP-Mel, CN-MelA, and GR-Mel cells | [16] |
| 1'-Hydroxymethyleugenol (α-ethenyl-3,4-dimethoxybenzenemethanol) | WST assay, SRB assay | Cytotoxic and genotoxic properties | V79 cells | [49] |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|-------------------------|-----------|
| L-Lactate assay | Cytotoxic effect | B6C3F1 mouse hepatocytes | [47] |
| UDS assay | Cytotoxicity and genotoxicity effects | F-344 rat hepatocytes | B6C3F1 mouse hepatocytes | [47] |
| Trypan-blue exclusion assay | Potential cytotoxic effects | Rat hepatocytes and SCC-4 cells | [47, 51, 54] |
| Flow cytometric assay | Induction of apoptosis of cells by involvement of mitochondria- and caspase-dependent signal pathway | SCC-4 cells | [51] |
| Western blotting analysis | Upregulation of the protein expression of Bax and Bid and downregulation of the protein levels of Bcl-2 (upregulation of the ratio of Bax/Bcl-2), resulting in cytochrome c release, promoted Apaf-1 level, and sequential activation of caspase-9 and caspase-3 in a time-dependent manner | SCC-4 cells | [51] |
| Real-time PCR | mRNA expressions of caspases 3, 8, and 9 | PC3 cells | [53] |
| MTT assay | Cytotoxic effect | Human BMFs | [52] |
| Western blot analysis | Activate NF-κB expression that may be involved in the pathogenesis of OSF and mediated by ERK activation and COX-2 signal transduction pathway | Human BMFs | [52] |
| Fura-2 as a probe assay | Induced a [Ca^{2+}], increase by causing Ca^{2+} release from the endoplasmic reticulum in a phospholipase C- and protein kinase C-independent fashion and by inducing Ca^{2+} influx | PC3 cells | [53] |
| Comet assay/(DAPI) staining | Induced apoptosis (chromatin condensation) and DNA damage | HL-60 cells | [51] |
| Flow cytometric analysis | Increased the production of reactive oxygen species (ROS) and Ca^{2+} and reduced the mitochondrial membrane potential | HL-60 cells | [51] |
| Western blotting analysis/confocal laser microscopy | Promoted the expression of glucose-regulated protein 78 (GRP78), growth arrest- and DNA damage-inducible gene 153 (GADD153), and activating transcription factor 6α (ATF-6α) | NK cells | [58] |
| Flow cytometric analysis | Promoted the levels of CD11b and Mac-3 that might be the reason for promoting the activity of phagocytosis; reduced the cell population such as CD3 and CD19 cells | NK cells | [58] |
| Ames test | Mutagenicity activity | Salmonella TA 98 | [59] |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|----------------------|--------------------------------------|-------------------------|-----------|
| Safrole-2-3-oxide 4-(2,3-epoxypropyl)-1,2- (methyleneoxy) | MTT assay | Produced toxicity in cells in a dose- and time-dependent manner | HepG2 cells | [56] |
| | Comet assay | Significant dose-dependent increase in the degree of DNA (strand breaks) | FVB mice | |
| | Comet assay | Increase in mean Comet tail moment in peripheral blood leukocytes and in the frequency of micronucleated reticulocytes | HepG2 cells | |
| | TUNEL assay | Activity of caspases 3, 8, and 9 | A549 cells | [58] |
| Myristicin (5-allyl-3-methoxy-1,2-methyleneoxybenzene) | Western blot assay | Cleavages of PARP, accompanied by an accumulation of cytochrome c and by the activation of caspase-3 | SK-N-SH cells | [60] |
| Estragole (1-allyl-4-methoxybenzene) | Induction of GST and QR | Induction of GST and QR in mouse livers | Four strains of mouse: A/JOlaHsd, C57BL/6NHsd, BALB/cAnNHsd, and CBA/JCrHsd | [61] |
| | Trypan-blue exclusion assay | Cytotoxic activity | Rat hepatocytes | [55] |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|-------------------------|-----------|
| Anethole [1-methoxy-4-(1-propenyl)benzene] | Trypan-blue assay | Cytotoxic activity | HeLa, rat hepatocytes cell | [21, 23, 55, 64] |
| | MTT assay | Cytotoxic activity | HT-1080, ML1-a cells | [63] |
| | Boyden-chamber assay | Reduced 40 and 85% of cells to invade into Matrigel | HT-1080 cells | [62] |
| | Gelatin zymography and RT-PCR analyses | Inhibitory effect of MMP-2 and MMP-9 and downregulate the expression of matrix metalloproteinases (MMPs) 2 and 9 and upregulate the gene expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) | HT-1080 cells | [62] |
| | Expression of MMPs, TIMPs, and uPA assays | Decreased mRNA expression of urokinase plasminogen activator (uPA) Suppressed the phosphorylation of AKT, extracellular signal-regulated kinase (ERK), p38, and nuclear transcription factor kappa B (NF-κB) | Rat hepatocytes MCF-7 cells ML1-a cells | [62] [63] |
| | Fluorometric analysis | Increases in the levels of ADP and AMP Estrogenic effect based on the concentrations of the hydroxylated intermediate, 4OHPB Suppress TNF-induced activation of the transcription factor AP-1, c-jun N-terminal kinase, and MAPK-kinase | Rat hepatocytes MCF-7 cells ML1-a cells | [62] [63] |
| | CCK-8 assay | | EAT cells in the paw of Swiss mice | [65] |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|-------------------------|-----------|
| trans-Anethole oxide (2R,3R)-2-(4-methoxyphenyl)-3-methyl-oxirane | Ames test | Mutagenic for *Salmonella* tester strains | *Salmonella typhimurium* strains TA1535, TA100, and TA98 | [67] |
| | Induction of hepatic tumors | Carcinogenic in the induction of hepatomas | B6C3F1 mice | |
| | Induction of skin papillomas | Carcinogenic in the induction of skin papillomas | CD-1 mice | |
| β-Asarone 1,2,4-trimethoxy-5-[(Z)-prop-1-enyl]benzene | SRB assay | Cytotoxic activity | A549, SK-OV-3, SK-MEL-2, and HCT15 cells | [70] |
| trans-Asarone oxide (1-propenyl-2,4,5-(trimethoxybenzene) | Ames test | Mutagenic for *Salmonella* tester strains | *Salmonella typhimurium* strains TA1535, TA100, and TA98 | [67] |
| | Induction of hepatic tumors | Carcinogenic in the induction of hepatomas | B6C3F1 mice | |
| | Induction of skin papillomas | Carcinogenic in the induction of skin papillomas | CD-1 mice | |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|-------------------------|-----------|
|                      |                       |                                      |                         | [71, 77, 78, 80, 84, 89] |
| **(E)-3-phenylprop-2-enal** | MTT assay | Cytotoxic activity                   | A375, HCT 116, MCF-7, P388, L-1210, 3LL, SNU-C5, HL-60, U-937, HCT 116, L1210 mouse, and Syrian hamster embryo cells | [71, 77, 78, 80, 84, 89] |
|                      | TRPA1 and TRPM8 gene expression | Reduce the proliferation of melanoma cells; this effect is independent of an activation of TRPA1 channels | A375, G361, SK-Mel-19, SK-Mel-23, and SK-Mel-28 cells HeLa, A549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15 cells | [77] |
|                      | Sulfurhodamine B assay | Cytotoxic activity                   | A375, G361, SK-Mel-19, SK-Mel-23, and SK-Mel-28 cells | [77] |
|                      | Amies test            | Not mutagenic                        |                         | [76] |
|                      | DTNB assay            | TrxR inactivation                    | HCT 116 cells           | [78] |
|                      | Western blot analysis | Nrf2-mediated upregulation of phase II enzymes, including TrxR induction |                         | [78] |
|                      | XTT assay             | Inhibitory effects on the growth of cells |                         | [80] |
|                      | Western blot analysis | Increase in the CD95 (APO-1/CD95) protein expression in Hep G2 cells |                         | [80] |
|                      | Trypan-blue assay     | Inhibited the proliferation of cells | PLC/PRF/5 cells         | [81] |
|                      | Flow cytometer analysis | Activation of proapoptotic |                         | [81] |
|                      | Western immunoblot analysis | Prevented the phosphorylation of JNK and p38 proteins |                         | [83] |
|                      | DAPI/Fluorometric method | Induced apoptosis in cells |                         | [83] |
|                      | Flow cytometry analysis | Induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release | P388, L-1210, 3LL, SNU-C5, HL-60, U-937, and HepG2 cells | [71] |
|                      | cis-DDP-induced       | Potentiates the inactivating effect of cis-DDP in all phases of the cell cycle | NHIK 3025 cells         | [82] |
|                      | NRU assay             | Induced the fragmentation of nuclei (Plate 2), which is typical for condensed apoptotic phenotype | Hep-2 cells              | [87] |
|                      | Genotoxicity assays—DNA repair test | Involve DNA damage as one of the factors involved in the mammalian cytotoxicity |                         | [88] |
|                      | LDH-cytotoxicity assay | Potent inhibitory effect against human hepatoma cell growth |                         | [92] |
|                      | Western blot analysis | JAK2-STAT3/STAT5 pathway may be important targets Decreased the protein levels of cyclin D1 and proliferative cell nuclear antigen (PCNA) but increased the protein levels of p27Kip1 and p21Waf1/Cip1 | HepG2 and Hep3B cells | [88] |
|                      | Flow cytometry analysis | Inducing apoptosis and synergizing the cytotoxicity of CIK cells | K562 cells               | [92] |
| Compound | Experimental protocol | Antitumoral activity and/or mechanism | Animal/cell line tested | Reference |
|----------|-----------------------|--------------------------------------|------------------------|-----------|
| **2′-Hydroxycinnamaldehyde** <sup>(3-acyloxy)-2-propenal</sup> | Spectral analysis | Induced an adaptive antioxidant response through Nrf2-mediated upregulation of phase II enzymes, including TrxR induction | S180 in mice | [89] |
| | MTT assay | Cytotoxic activity | NIH/3T3 cells | [90] |
| | Lymphoproliferation—Con A, LPS, or PMA plus ionomycin | Inhibit the lymphoproliferation and induce a T-cell differentiation from CD4CD8 double positive cells to CD4 or CD8 single positive cells | Mice splenocytes | [74] |
| | Flow cytometry analysis | Capability to block the cell growth and stimulate a differentiation to mature cell | | |
| | IgM-secreting B cells to SRBC | Decreased level of IgM to be due to the lower level of B-cell proliferation | Balb/c mice | |
| **Cinnamic acid [(E)-3-phenylprop-2-enic acid]** | ELISA | Inhibits proliferation and DNA synthesis | Caco-2 cells | [79] |
| | Radioimmunoassay | Decreased intracellular cAMP levels | U14 cells | [92] |
| | Flow cytometry analysis | Influence on the tumor cell cycle. G2-M period shortened, cell cycle lengthened, and cell proliferation inhibited | NHIK 3025 cells | [91] |
| | cis-DDP-induced | Potentiated the inactivating effect of cis-DDP in all phases of the cell cycle | HL-60, A549, PC3, Du145, LN-CaP, A172, U251, SKMEL28, and A375 cells | [93, 94] |
| | Trypan-blue assay | Anticancer activity | Human osteogenic sarcoma cells | [95] |
| | Flow cytometry analysis | Inhibition and induced-differentiation on human osteogenic sarcoma cells | | |
| | MTT assay | Cytotoxic activity | HepG2 cells | [97] |
| | Spectrophotometer | Higher antioxidant capacity | Mac Coy cells | [96] |
| | NRU assay | Cytotoxic activity | EHV-1 | [98] |
| | MTT assay | Antiviral activity | | |
| **Hydroxychavicol** 4-prop-2-enediol | Trypan-blue assay | Cytotoxic activity | Decrease in cell viability, accompanied by losses of ATP, GSH; increase in GSSG, ROS, and MDA levels | Rat hepatocytes | [54] |
| | Waters chromatograph | | | |
| **1′-Acetoxychavicol acetate** (1S)-1-[4-(acetoxy)phenyl]prop-2-en-1-yl acetate | Indirect immunofluorescent method/EBV activation | Inhibiting the generation of anions during tumor promotion | Raji cells | [100] |
| | Trypan-blue exclusion assay | Cytotoxic activity | RPMI8226, U266, and IM-9 cells | |
| | Flow cytometry | Induced caspases 3, 9, and 8 activities | | |
| | Western blot analysis | TNF-α-induced apoptosis | | |
| | ELISA | Downregulation of NF-κB activity | | |
| | In vivo assay | TNF-α-induced apoptosis | | |
| | | Anticancer effects with no toxic effects | | NOD/SCID mouse | [99] |
isoeugenol showed carcinogenic effects, causing increased incidence of rarely occurring thymoma and mammary gland carcinoma. There was no evidence of carcinogenic activity due to isoeugenol in female F344/N rats. However, there was clear evidence of carcinogenic activity due to isoeugenol in male B6C3F1 mice, including increased incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma with carcinoma. Carcinogenic activity due to isoeugenol in female B6C3F1 mice was observed in the form of increased incidence of histiocytic sarcoma. Exposure to isoeugenol resulted in nonneoplastic lesions of the nose in male and female rats, of the kidney in female mice, and of the nose, forestomach, and glandular stomach in mice of both sexes [50]. However, methyleugenol is minimally cytotoxic for hepatocytes and leukemia cells compared to eugenol [48, 49]. The structural similarity of these substances with eugenol stimulates advances in pharmacological studies to explore their therapeutic potential in cancer treatment.

2.3. Safrole, Safrole-2′,3′-oxide, and Myristicin. Safrole is an important food-borne phytotoxin found in many natural products, such as oil of sassafras, anise, basil, nutmeg, and pepper. Safrole is cytotoxic against human tongue squamous carcinoma [51], primary human buccal mucosal fibroblasts [52], prostate cancer [53], rat hepatocytes [54], and leukemia [51] and shows genotoxic activity [55, 56].

Safrole induced apoptosis in human tongue squamous carcinoma SCC-4 cells by mitochondria- and caspase-dependent signaling pathways. Safrole-induced apoptosis was accompanied by upregulation of Bax and Bid and downregulation of Bcl-2, which increased the ratio of Bax/Bcl-2, resulting in cytochrome c release, increased Apaf-1 levels, and sequential activation of caspase-9 and caspase-3 in a time-dependent manner [51]. In A549 human lung cancer cells, safrole activated caspases 3, 8, and 9 [57]. In rat hepatocytes cells, safrole induced cell death by loss of mitochondrial membrane potential and generation of oxygen radical species, which were assayed using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) [54].

Fan and collaborators [58] showed that safrole promoted the activities of macrophages and NK cells in BALB/c mice. While promoting macrophage phagocytosis, safrole increased abundance of cell markers such as CD11b and Mac-3. Additionally, NK cell cytotoxicity was remarkably suppressed in mice treated with safrole, as were levels of cell markers for T cells (CD3) and B cells (CD19). Safrole was also cytotoxic against primary human buccal mucosal fibroblasts (BMFs) [52]. Ni and collaborators [52] demonstrated that safrole increased NF-κB expression, which may have been involved in the pathogenesis of oral submucous fibrosis. NF-κB expression induced by safrole in fibroblasts may be mediated by ERK activation and the COX-2 signal transduction pathway.

A study by Chang and collaborators [53] investigated the effect of safrole on intracellular Ca\(^{2+}\) mobilization and viability of human PC3 prostate cancer cells. Cytosolic free Ca\(^{2+}\) levels ([Ca\(^{2+}\)]\(_i\)) were measured using fura-2 as a probe. Safrole increased [Ca\(^{2+}\)], by causing Ca\(^{2+}\) release from the endoplasmic reticulum in a phospholipase C- and protein kinase C-independent manner, which decreased cell viability in a concentration-dependent manner. In HL-60 leukemia cells, safrole promoted the expression of glucose-regulated protein 78 (GRP78), growth arrest- and DNA damage-inducible gene 153 (GADD153), and activating transcription factor 6α (ATF-6α) [51]. In the unscheduled DNA synthesis (UDS) assay described by Howes and collaborators [55], safrole exhibited genotoxic activity in freshly isolated rat hepatocyte primary cultures.

Safrole-2′,3′-oxide (SAFO) is a reactive electrophilic metabolite of safrole. SAFO is the most mutagenic metabolite of safrole that has been tested in the Ames test, but data on the genotoxicity of SAFO in mammalian systems is scarce. SAFO induced cytotoxicity, DNA strand breakage, and micronuclei formation in human cells in vitro and in mice [56]. In addition, safrole produced mutagenicity in Salmonella TA 98 and TA 100 in the Ames test [59].

Myristicin (1-allyl-3,4-methylenedioxy-5-methoxybenzene) is an active constituent of nutmeg, parsley, and carrot. A study by Lee and collaborators [60] investigated the cytotoxic and apoptotic effects of myristicin on human neuroblastoma SK-N-SH cells. Apoptosis triggered by myristicin was caused by cleavage of PARP, which was accompanied by accumulation of cytochrome c and activation of caspase-3. These results suggested that myristicin induced cytotoxicity in human neuroblastoma SK-N-SH cells by an apoptotic mechanism [60].

Ahmad and collaborators [61] investigated the effect of myristicin on activity of glutathione S-transferase (GST) and NADPH:quinone oxidoreductase (QR) in four mouse strains. The authors showed that activity of GST and QR was significantly increased in the livers of all four mouse strains, GST activity was increased in the intestine of three out of four strains, and QR activity was significantly increased in the lungs and stomachs of three out of four strains. Thus myristicin, which is found in a wide variety of herbs and vegetables, shows strong potential as an effective chemoprotective agent against cancer.

Safrole, safrole-2′,3′-oxide, and myristicin are bioactive substances in antitumor models that can be used as starting materials for the preparation of derivatives with improved pharmacological profile.

2.4. Estragole, Anethole, and trans-Anethole Oxide. Estragole has been isolated from essential oils of Artemisia dracunculus and Leonotis ocymifolia. Howes and collaborators [55] demonstrated the genotoxic activity of estragole via UDS assay, in which estragole induced dose-dependent increases in UDS up to 2.7 times that of the control in rat hepatocytes in primary culture.

Anethole (1-methoxy-4-(1-propenyl)benzene) occurs naturally as a major component of essential oils from fennel and star anise and is also present in numerous plants such as dill, basil, and tarragon [62]. Anethole had a cytotoxic effect on fibrosarcoma tumor [63], breast cancer [63], hepatocytes [55, 64], cervical carcinoma [21, 23], and Ehrlich ascites tumor [65], as well as an anticarcinogenic effect and a lack of clastogenic potential [65].
Chainy and collaborators [66] reported that anethole reduced apoptosis by inhibiting induction of NF-κB, activator protein 1 (AP-1), c-jun N-terminal kinase (JNK), and mitogen-activated protein kinase kinase (MAPKK) by tumor necrosis factor (TNF). Choo and collaborators investigated the antimitotic activity of anethole [63] and showed that anethole inhibited proliferation, adhesion, and invasion of highly metastatic human HT-1080 fibrosarcoma cells. Anethole also inhibited the activity of metalloproteinases (MMP-2 and MMP-9) and increased the activity of MMP inhibitor TIMP-1 [63]. Nakagawa and Suzuki [62] showed that anethole induced a concentration- and time-dependent loss of cell viability in isolated rat hepatocytes, which was followed by decreases in intracellular levels of ATP and total adenine nucleotide pools. Howes and collaborators [55] demonstrated that anethole did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes in primary culture. In Ehrlich ascites tumor-bearing mice, anethole increased survival time and reduced tumor weight, tumor volume, and body weight [65].

Anethole is metabolized through 3 pathways: O-demethylation, α-hydroxylation followed by side chain oxidation, and epoxidation of the 1,2-double bond. The cytotoxicity of trans-anethole oxide in rat hepatocytes has been shown to be due to its metabolism to epoxide [67]. In addition, trans-anethole oxide produced a positive result in the Salmonella mutation assay and induced tumors in mice. These results suggest that epoxidation of the side chain of anethole in vivo could be a carcinogenic metabolic mechanism. Kim and collaborators [67] found that trans-anethole oxide is more toxic to animals than trans-anethole and was mutagenic in point mutation and frameshift mutation Ames test models. trans-Anethole did not induce hepatomas in male B6C3F1 mice, but the highest dose of trans-anethole oxide tested (0.5 μmol/g) significantly increased the incidence of hepatomas.

2.5. Asaraldehyde, β-Asarone, and trans-Asarone Oxide. Acorus gramineus (Araceae), which is distributed throughout Korea, Japan, and China, has been used in Korean traditional medicine for improvement of learning and memory, sedation, and analgesia [68]. Several pharmacologically active compounds, such as β-asarone, α-asarone, and phenylpropenes, have been reported from this rhizome [69]. Park and collaborators [70] investigated asarone and asaraldehyde and showed minimal cytotoxicity (IC₅₀ < 30 μM) in the SRB assay using 4 human tumor cell lines: A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cell), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cell). trans-Asarone oxide, prepared from trans-asarone and dimethyldioxirane, induced hepatomas in B6C3F1 mice and skin papillomas in CD-1 mice and was mutagenic for Salmonella strains [67].

2.6. Cinnamaldehyde, 2’-Hydroxycinnamaldehyde, and Cinnamic Acid. Cinnamaldehyde is a bioactive compound isolated from the stem bark of Cinnamomum cassia and has been widely used in folk medicine for its anticancer [71], antibacterial [72], antimutagenic [73], and immunomodulatory effects [74], as well as to remedy other diseases [75]. The cytotoxic activity of cinnamaldehyde has been confirmed in melanoma [76, 77], the colon [76, 78, 79], breast cancer [78], hepatic tumor [80, 81], leukemia [71, 82, 83], cervical carcinoma [76, 83] the lung, the ovary, the central nervous system [76], lymphoma, mouse leukemia [76, 84], mouse lung carcinoma [71], lymphocytes [74], hepatocytes [85], embryo cells [86], and larynx carcinoma [87]. Its genotoxicity has been confirmed in vitro [87]. Cinnamaldehyde also had genotoxic effects against SA7-transformed Syrian hamster embryo cells [86].

Ng and Wu [80] showed that cinnamaldehyde induced lipid peroxidation in hepatocytes isolated from male Sprague-Dawley rats with glutathione depletion. Adding NADH generators, for example, xyliol, prevented cytotoxicity induced by cinnamaldehyde, but decreasing mitochondrial NAD⁺ with rotenone markedly increased cinnamaldehyde cytotoxicity. The authors showed that cinnamaldehyde-induced cytotoxicity and inhibition of mitochondrial respiration were markedly increased by ALDH inhibitors and in particular by cyanamide [80].

Chew and collaborators [78] used flow cytometric analysis to show that 80 μM of cinnamaldehyde caused cell cycle arrest at the G₂/M phase in HCT 116 cells and induced cleavage of caspase-3 and PARP. It has also been proposed that cinnamaldehyde induced apoptosis by ROS release with TrxR-inhibitory and Nrf2-inducing properties [78]. Ka and collaborators [71] demonstrated that cinnamaldehyde-induced ROS-mediated mitochondrial permeability and cytochrome c release in human leukemia cells (HL-60).

Using hepatoma cells, Wu and collaborators [81] demonstrated that cinnamaldehyde upregulated Bax protein, down-regulated Bcl-2 and Mcl-1, and caused Bid to cleave upon the activation of caspase-8. These events consequently led to cell death. JNK, p38, and ERK were activated and phosphorylated after cinnamaldehyde treatment in a time-dependent manner, which suggested that apoptosis was mediated by activation of proapoptotic Bcl-2 family (Bax and Bid) proteins and MAPK pathways [81]. Cinnamaldehyde can also activate TRPA1 expression in melanoma cells [77].

Cinnamaldehyde caused a time-dependent increase in CD95 (APO-1/CD95) protein expression in HepG2 cells (human hepatoma), while also downregulating antiapoptotic proteins (Bcl-XL) and upregulating proapoptotic (Bax) proteins in a time-dependent manner [80]. Preincubation of HepG2 cells with cinnamaldehyde effectively inhibited the expression of Bax, p53, and CD95, as well as the cleavage of PARP. This pretreatment also prevented downregulation of Bcl-XL [80]. Using the HepG2 and Hep3B human hepatoma cancer cell lines, Chuang and colleagues [88] demonstrated that cinnamaldehyde had a potent inhibitory effect against human hepatoma cell growth. They observed that the JAK2/STAT3/STAT5 pathway might be an important target of cinnamaldehyde. Cinnamaldehyde also altered apoptotic signaling. Cinnamaldehyde significantly decreased protein levels of cyclin D1 and proliferative cell nuclear antigen (PCNA) but increased the protein levels of p27Kip1 and p21Waf1/Cip1 [86]. In an assay of thioredoxin reductase (TrxR) action, cinnamaldehyde showed a TrxR inactivation effect.
effects in vivo [90]. Immune cell infiltration into hepatic tissues was increased long-term immunostimulating effect on T cells, because ras12V transgenic mice, where they probably produced a demonstrated on hepatocellular carcinoma formation in H-chemopreventive effects of cinnamaldehyde derivatives were tive, was studied for its immunomodulatory effects. The treatment splenocytes. Decreased IgM produced by cinnamaldehyde IgM level was depressed in the culture supernatants of that could contribute to its cytotoxicity [89]. Furthermore, cinnamaldehyde had an antitumor effect in Sarcoma 180-bearing BALB/c mice and a protective effect on immune function [89].

2'-Hydroxycinnamaldehyde, a cinnamaldehyde derivative, was studied for its immunomodulatory effects. The chemopreventive effects of cinnamaldehyde derivatives were demonstrated on hepatocellular carcinoma formation in Harasi2V transgenic mice, where they probably produced a long-term immunostimulating effect on T cells, because immune cell infiltration into hepatic tissues was increased [90].

2'-Hydroxycinnamaldehyde has immunomodulatory effects in vivo, but in vitro studies showed that secreted IgM level was depressed in the culture supernatants of splenocytes. Decreased IgM produced by cinnamaldehyde treatment in vitro appeared to be due to lower levels of B-cell proliferation, rather than direct inhibition of IgM production [74]. Koh and collaborators [74] also demonstrated that cinnamaldehyde induced T-cell differentiation from CD4CD8 double positive cells to CD4 or CD8 single positive cells.

Cinnamic acid occurs throughout the plant kingdom and particularly in flavor compositions and products containing cinnamon oil [91]. Cinnamic acid inhibited proliferation of uterocervical carcinoma [92], leukemia [93], colon adenocarcinoma [79], glioblastoma, melanoma, prostate, lung carcinoma [94], osteogenic sarcoma [95] cells, Mac Coy cells [96], Hep G2 cells [97], and kidney epithelial (VERO) cells [98].

Cinnamic acid had an inhibitory effect on uterocervical carcinoma (U14) cells in mice, causing tumor cell apoptosis [92]. In vitro assay of U14 cells demonstrated a shortened G2-M period, lengthened cell cycle, and inhibited cell proliferation, which supported the conclusion that cinnamic acid influenced tumor cell cycle [92].

Ekmekcioglu and collaborators [79] showed that cinnamic acid inhibited proliferation and DNA synthesis of Caco-2 (human colon) cells. Treatment with cinnamic acid modulated the Caco-2 cell phenotype by dose-dependently stimulating sucrase and aminopeptidase N activity, while inhibiting alkaline phosphatase activity. In melanoma cells cinnamic acid induced cell differentiation with morphological changes and increased melanin production. Cinnamic acid reduced the invasive capacity of melanoma cells and modulated expression of genes implicated in tumor metastasis (collagenase type IV and tissue inhibitor metalloproteinase 2) and immunogenicity (HLA-A3, class-I major histocompatibility antigen) [94].

### Figure 1: Possible mechanisms of action from phenylpropanoids antitumoral activity.

| Phenylpropanoids                                      | Possible mechanisms of action |
|-------------------------------------------------------|------------------------------|
| • Prevented the phosphorylation of JNK and p38 proteins | Promoted the levels of CD11b and Mac-3 that might be the reason for promoting the activity of phagocytosis |
| • Deregulation of the E2F family of transcription factors | Induced caspases 3, 9, and 8 activities |
| • Upregulation of p53 expression with a concomitant increase in p21WAF1 levels | Upregulation of phase II enzymes |
| • Upregulate the gene expression of tissue inhibitor of TIMP-1 | Inhibition of topoisomerase II |

| Phenylpropanoids                                      | Possible mechanisms of action |
|-------------------------------------------------------|------------------------------|
| • Proliferative cell nuclear antigen (PCNA) but increased protein levels of p27kip1 and p21Waf1/Cip1 | Downregulated the expression of MMP-2 and -9 |
| • Protect cells via inhibition of xanthine oxidase activity and lipid peroxidation | Induced a [Ca2+]i, increase by causing Ca2+ release |
| • TrxR induction                                      | Decreased cellular ATP level |
| • Reduced the nicotine-induced ROS, NO generation, and iNOSII expression | Increases in the levels of ADP and AMP |
| • Downregulated the expression of Bcl-2, COX-2, and IL-β | Apoptotic manifestations via phospho-ser 15-p53 into mitochondria |
| • Increase in LDH release                             | Inhibition of the proliferation associated genes c-Myc and H-ras |
| • Production of ROS                                    | Depleted the level of intracellular glutathione |
| • Reduced the cell population such as CD3 and CD19     | Hemolytic activity |
| • Increase in the CD95 (APO-1/CD95) protein expression | Suppressed the phosphorylation of AKT, extracellular signal-regulated kinase (ERK) and p38 |
| • Decreased the protein levels of cyclin D1            | Antioxidative activity/stimulation the production of superoxide (O2-)
| • Transcriptional activity of E2F1                     | Induced an adaptive antioxidant response through Nrf2-mediated upregulation of phase II enzymes, including TrxR induction |

| Phenylpropanoids                                      | Possible mechanisms of action |
|-------------------------------------------------------|------------------------------|
| • Increased in LDH release                            | Inhibition of topoisomerase II |
| • Protect cells via inhibition of xanthine oxidase activity and lipid peroxidation | Apoptotic manifestations via phospho-ser 15-p53 into mitochondria |
| • TrxR induction                                      | Inhibition of the proliferation associated genes c-Myc and H-ras |
| • Reduced the nicotine-induced ROS, NO generation, and iNOSII expression | Depleted the level of intracellular glutathione |
| • Downregulated the expression of Bcl-2, COX-2, and IL-β | Hemolytic activity |
| • Increased in LDH release                            | Suppressed the phosphorylation of AKT, extracellular signal-regulated kinase (ERK) and p38 |
| • Production of ROS                                    | Antioxidative activity/stimulation the production of superoxide (O2-)
| • Reduced the cell population such as CD3 and CD19     | Induced an adaptive antioxidant response through Nrf2-mediated upregulation of phase II enzymes, including TrxR induction |

| Phenylpropanoids                                      | Possible mechanisms of action |
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| • Reduced the cell population such as CD3 and CD19     | Induced an adaptive antioxidant response through Nrf2-mediated upregulation of phase II enzymes, including TrxR induction |
Using *in vivo* and *in vitro* assays, Zhang and collaborators (2010) [92] showed that cinnamic acid influenced the cell cycle of uterocervical carcinoma cells (U14); the \(\text{G}_2\)-\(\text{M}\) period was shortened, cell cycle was lengthened, and cell proliferation was inhibited. Cinnamic acid also induced differentiation of human osteogenic sarcoma cells and caused a higher percentage of cells in S phase [95].

2.7. Hydroxychavicol and 1'-Acetoxychavicol Acetate. Hydroxychavicol (1-allyl-3,4-dihydroxybenzene) is a major component in *Piper betle* leaf, which is used for betel quid chewing in Asia, and is also a major metabolite of safrole, which is the main component of sassafras oil, in rats and humans. A study by Nakagawa and collaborators [54] demonstrated the biotransformation and cytotoxic effects of hydroxychavicol in freshly-isolated rat hepatocytes. In hepatocytes pretreated with diethyl maleate or salicylamide, hydroxychavicol-induced cytotoxicity was enhanced and was accompanied by a decrease in the formation of conjugates and inhibition of hydroxychavicol loss.

Other studies indicate that mitochondria are the target organelles for hydroxychavicol, which induces cytotoxicity through mitochondrial failure related to mitochondrial membrane potential at an early stage, and lipid peroxidation through oxidative stress at a later stage. Furthermore, the onset of cytotoxicity depends on the initial and residual concentrations of hydroxychavicol, rather than its metabolites.

1'-Acetoxychavicol acetate is obtained from the rhizomes of *Languas galanga* (Zingiberaceae), a traditional condiment in Thailand. Recent studies have revealed that 1'-acetoxychavicol acetate has potent chemopreventive effects against rat oral carcinomas and inhibits chemically induced tumor formation and cellular growth of cancer cells. 1'-Acetoxychavicol acetate inhibited NF-\(\kappa\)B and induced apoptosis of myeloma cells *in vitro* and *in vivo*. Therefore, 1'-acetoxychavicol acetate is a novel NF-\(\kappa\)B inhibitor and represents a new therapy for the treatment of multiple myeloma patients [99]. The isolation and identification of 1'-acetoxychavicol acetate, an inhibitor of xanthine oxidase, may induce antitumor activity by inhibiting generation of anions during tumor promotion [100] (Figure 1).

### Abbreviations

#### Cell Lines

- 3LL: Mouse lung carcinoma
- A172: Human malignant glioblastoma
- A375: Melanoma
- A549: Lung adenocarcinoma
- BMFs: Primary human buccal mucosal fibroblasts
- Caco-2: Human colon adenocarcinoma
- CD11b: Monocytes
- CD19: B cells
- CD3: T cells
- CEM: Acute T lymphoblastoid leukemia
- CN-Mel: Melanoma
- DU-145: Androgen-insensitive prostate cancer
- F344: Hepatocytes
- G361: Melanoma
- GR-Mel: Melanoma
- HCT-15: Colon tumor
- HeLa: Human cervical carcinoma
- Hep3B: Human hepatoma cancer
- HepG2: Human hepatoma
- HGF: Human gingival fibroblasts
- HL-60: Human promyelocytic leukemia
- HSC-3: Human oral cancer cells
- HSG: Human submandibular gland carcinoma
- HT-1080: Human fibrosarcoma tumor
- K-562: Human chronic myelogenous leukemia
- KB: Oral squamous carcinoma
- L-1210: Mouse leukemia
- LCM-Mel: Melanoma
- LCP-Mel: Melanoma
- LN-CaP: Prostate cancer
- Mac-3: Macrophages
- MCF-7 gem: Human breast adenocarcinoma (resistant to gemcitabine)
- MCF-7: Human breast adenocarcinoma
- ML-1: Human myeloblastic leukemia
- NHIK 3025: Human cervical carcinoma
- P388: Mouse leukemia
- P-815: Murine mastocytoma
- PC-3: Human prostate cancer
- PLC/PRF/5: Human hepatoma
- PNP-Mel: Melanoma
- Raw 264.7: Mouse leukemic monocyte macrophage
- S180: Sarcoma 180
- SbCl2: Primary melanoma
- SCC-4: Tongue squamous carcinoma
- SK-Mel-19: Melanoma
- SK-MEL-2: Skin melanoma
- SK-MEL-23: Melanoma
- SK-MEL-28: Melanoma
- SK-N-SH: Neuroblastoma
- SK-OV-3: Ovarian cancer
- SNU-C5: Human colon cancer
- U14: Uterocervical carcinoma
- U251: Human malignant glioblastoma
- U-937: Human histiocytic lymphoma
- uPA: Urokinase plasminogen activator
- WM1205Lu: Metastatic melanoma
- WM266-4: Melanoma
WM3211: Primary radial growth phase melanoma
WM98-1: Primary vertical growth phase melanoma
XF-498: Central nervous system.

Tests
AFC: Antibody forming cell
ALDH: Aldehyde dehydrogenase
Ames test: Biological assay to assess the mutagenic potential of chemical compounds
Boyden-chamber assay: Evaluation of tumor cell invasion in vitro
c-AMP: Cyclic adenosine monophosphate
CAS: Chromosomal aberrations
CCK-8: Cell Counting Kit-8, a sensitive colorimetric assay
CDFHDA: 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate
Comet assay: Single-cell gel electrophoresis
Con A: Concanavalin
DAPI: 4',6-Diamidino-2-phenylindole
DCFH: Dichlorofluorescein
DEM: Diethyl maleate
DMBA: 7,12-Dimethylbenz[a]anthracene
DPPH: 1,1-Diphenyl-2-picrylhydrazyl
DTNB: 5,5'-Dithiobis-(2-nitrobenzoic acid)
EBV: Epstein-Barr virus
EHV-1: Herpes virus 1
ESR: Electron spin resonance spectroscopy
GSSG: Oxidized glutathione
GST: Glutathione S-transferase
LDH: Lactate dehydrogenase
LPS: Lipopolysaccharide
MDA: Malondialdehyde
MMP: Matrix metalloproteinase
MTT: [3(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide]
NF-κB: Nuclear factor-kappa B
NRU assay: Neutral red uptake
p21WAF1: Cyclin-dependent kinase inhibitor CDKN1A
PARP: Poly (ADP-ribose) polymerase
PBS: Phosphate-buffered saline
PCR: Polymerase chain reaction
PMA: Phorbol 12-myristate-13-acetate plus ionomycin
QR: Quinone oxidoreductase
SRB: Sulforhodamine B
SRBC: Sheep red blood cells
TBA: Test in the aqueous phase

TBARS: Thiobarbituric acid reactive substances
TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling
UDS assay: Unscheduled DNA synthesis
V-FITC assay: Apoptosis detection kit
WST: Tetrazolium salt
XTT: 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.

Conflict of Interests
The authors declare no conflict of interests.

Acknowledgments
This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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