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*Review Article*

# Anticancer Effects of *Melissa officinalis*: A Traditional Medicine

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**Abstract**

*Melissa officinalis (M. officinalis)* is an herbal-based plant from the family of Lamiaceae and native to Europe and the Mediterranean region, widely used to cure various cancers. Phytochemical investigations proved different compounds such as polyphenolic compounds, flavonoids, and essential oil in the stem and leaves of *M. officinalis* as main ingredients contributing to different antitumor activity, including antiproliferation and antioxidant antiangiogenetic, antimigratory, antiapoptotic, and change in cell cycle profile of cancer cells. Herbal formulations with colorful ingredients use several types of these mentioned biological processes to display synergistic cancer treatment activities. *M. officinalis* extracts a wide range from water to ethanol using varied mechanisms to reduce the viability of cancer cells. Hence, scientists are currently interested in evaluating these extracts based on the medical plant to minimize the adverse effects of conventional anti-cancer drugs and discover these mechanisms to pave the way for future studies. This review aimed to discuss the recent studies that *M. officinalis* have used as an anti-cancer agent to investigate its potential effect on several types of cancer. Therefore, after a short introduction of *M. officinalis*, we will explain the several biological processes by which *M. officinalis* exert an anti-cancer effect.

## Introduction

Cancer is a leading cause of death arising from genetic alterations, abnormal, uncontrolled proliferation, and the spread of cells that led to tissue destruction.[1,2] Lymphatic vessels and the bloodstream help cancer cells to spread to distant organs.[3,4] Disturbances in two oncogenes and tumor suppressor genes are responsible for growth and privation from developing, respectively. There is no defined risk factor for specific cancers; however, excessive tobacco and alcohol consumption, environmental pollutant, lifestyles, and exposure to infectious agents and radiations have a severe role in cancer disease development.[5] Unintentional weight loss, excessive tiredness, or fever are important appearance symptoms for recognition of cancer disease. When it comes to categorizing cancer, the type of cell that tumor emerges determines the cancer name. For instance, carcinoma refers to cancer that appears from the epithelial cell lining, muscle bones, cartilage, and connective tissue cancer cause by mesodermal cell lining called sarcoma. Cancer arises from bone marrow cells called leukemia and cancer involving the immune system called lymphoma.[5,6] Due to the increased incidence of cancer, researchers are encouraged to find new and more effective cancer therapy methods. Conventional clinical therapies for cancer disease include chemotherapy, radiation, immunomodulation, and surgery, which have high morbidity and mortality rate. For example, chemotherapy as a common way to treat cancer resulted in severe toxicity and cancer treatment failure. Therefore, new cancer treatment methods are still needed.[7,8] Medicinal plants are considered as an alternative option to reduce synthetic chemotherapeutic drugs.[9] Therefore, investigation on plant kingdoms with high potential as a cancer treatment and prevention compound has earned great attention in the last few years.[7,10] P*odophyllum peltatum*, *Taxus baccata*, *Camptotecha accuminata*, and *Vinca rosea* are several plant species recognized in clinical trials and marketed for breast cancer therapy. Medicinal plants are composed of various chemicals that have therapeutic value and display physiological impacts on the human body.[10,11] Nowadays, medical plants make strong participating in modern therapy, i.e., one hundred new plant-based drugs have been introduced to the drug market in the USA, including vincristine, deserpidine, reserpine, and vinblastine. Nevertheless, using synthetic chemicals to treat cancer, plant-based drugs decreased up to one quarter in the middle of the 20$^{th}$ century.[5] Fortunately, herbs are currently used as complementary medicine because these plants have different natural

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products that are more effective with little or no adverse effects than synthetic chemicals.[12] Plants are enriched with a different type of anti-cancer agent, generating 60% of all anti-cancer agents. In contrast, a large proportion of anti-cancer component-based medical plants needs to be discovered and evaluated.[5] Herbal formulations consist of dietary bio-factors, phytochemicals, chemopreventive and pharmacological ingredients that display synergistic activities. Thus, various plant mixtures or plant extracts show more therapeutic and preventive activity due to their multiple intervention strategies compared to single agents discovered from a defined plant. Studies have shown that the crude extract of pharmacological properties can be lost after isolating a particular plant extract component. Therefore, multitargeting would be the most effective and rational method for eliminating heterogeneous cancer cells.[1] Besides, it is proved that diets are the most vital factors that could induce or prevent cancer development. As a part of the human diet, these natural resources used in normal life in different cultures are non-toxic to the human body and control multiple signaling pathways.[13] Hence, in some economically unprivileged countries, plant extracts consisting of micronutrients, such as antioxidants with medicinal properties to scavenge free radicals, may prevent or modulate cells from hazardous effects.[10] *M. officinalis* L. (Lamiaceae) (Lemon balm) is a medicinal plant, which its leaves are used as herbal tea to relieve anxiety, improve indigestion and overcome sleeplessness. Several studies have proven the antitumor effect of *M. officinalis*, attributed to special components.[14,15] For example, polyphenolic compounds of *M. officinalis* are known as a potent chemopreventive agent preventing tumor formation and subsequently hinder cancer progression. Easy to access in high proportions and safety, contribution to health is a rather important feature of *M. officinalis* led to priority in consumption compared to synthetic drugs.[16,17] In this article, we will discuss the application of *M. officinalis* as a cancer preventive agent in cancer treatment. Anti-migration, antiangiogenics, antiproliferation, antiapoptotic are the most vital anti-cancer effects of *M. officinalis* by which influence cancer cells, and we will discuss these mechanisms in detail through this review.

## *M. officinalis* Application in Biomedical Sciences

It is shown that more than 20,000 plant species have the potential to be considered as a discovery of drugs in traditional medicine worldwide.[18] Lemon balm *(M. officinalis* L., Lamiaceae), a member of the family Lamiaceae known as Lemon Balm, garden balm, honey balm, and bee balm, is one of the recognized perennial bushy herbs used in South Europe, Western Asia, North Africa, and East Mediterranean regions as a food and beverage additive to emit a distinct fragrant lemon odor.[19] Although dumb wasteland and areas ranging from sea to the mountains are home to *M. officinalis*, sandy and scrubby areas are the best places where this plant grows naturally. This upright plant has hairy, soft, and heart-shaped leaves with white or pale pink flowers blossoming in the summer.[12] Besides, *M. officinalis* is more adaptable to harsh environmental conditions due to a hairy and lateral root system, i.e., the upside part of the plant dies off in winter, and new stems regrow at the begging of spring.[20] As a remedy in traditional medicine, it treats several disorders, including hypoglycemia, rheumatism, hypnotist, headaches, gastrointestinal disorders, nervous disturbance such as anxiety and surgical dressing, cardiovascular, hypolipidemia.[14]

Furthermore, it improves immunoprotection, hepatoprotection, nephroprotection, carminative, diaphoretic, sedative, and strengthens memory.[21] Dried forms such as extracts and syrups or fresh leaves and the top aerial section of the plant are the parts that are used as medicine. However, a spray of this plant cures crooked necks, toothache, baldness, pregnancy sickness and stopped the blood of the wound.[22] The phytochemical compounds responsible for pharmacological and therapeutically features isolated from their crude extracts are essential oils, flavonoids, tannins, terpenes (monoterpenes, and sesquiterpenes), and polyphenolic compounds like luteolin, caffeic acid derivatives,[12] hesperidin, naringin, coumarinic acid, and rosmarinic acid, volatile compounds such as geranial, neral, geraniol, and citronellal, trimeric compounds, luteolin-7-O-glucoside, rhamnocitrin, and triterpenes such as oleanolic and ursolic acids.[19,23] However, phenolics content of the plant, particularly rosmarinic acid (R.A), can be associated with antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, spasmolytic, and antitumor activities.

Moreover, R.A is an identified biomarker for quality control of WHO on selected medicinal plants.[19] Essential oils called secondary metabolites of plants and phytocomplexes, show different pharmacological and biological effects such as spasmolytic, antimicrobial, antioxidative hepatoprotective effect, and lower lipids content liver tissues to reduce total cholesterol increase glutathione level in the tissues.[13] Isogeraniol, geraniol acetate, caryophyllene oxide, citronellal, and nerol acetate are dominant compounds of essential oil in the leave of *M. officinalis*, resulting in distinctiveness aroma, cancer prevention, and treatment properties of essential oils.[12] For instance, citral, citronellal, and geraniol are responsible for the antiproliferative activity of the essential oils. Besides, essential oils increase the cytotoxicity effect of several anti-cancer drugs, including Paclitaxel and Docetaxel, which reduced the required drug concentration with the same efficacy in cancer treatment.[1] The intracellular level of oxygen radicals is combated via the citral component, which brings about cellular stress modulation and consequently drops in cancer cell proliferation and cell death. Induction of caspase activity is another effect of citral cells triggering apoptosis in several cancer cells. Besides, vitamins E, C, and glucose are another critical component of *M. officinalis*, an inactivator of free radicals. In general, several exogenous factors, including climate,

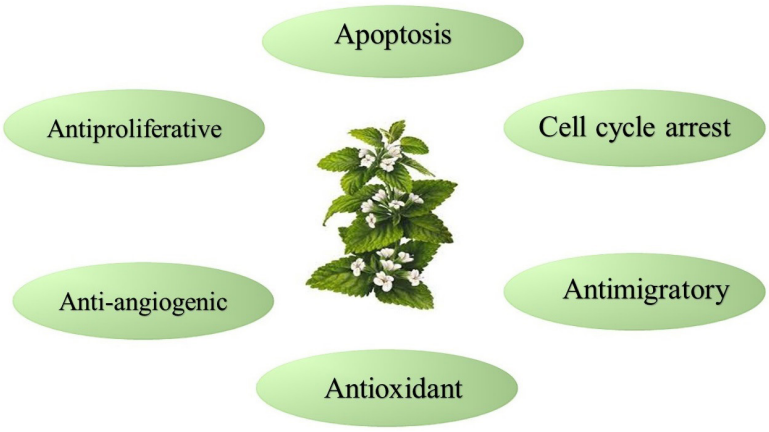


**Figure 1.** Schematic illustration of mechanisms by which *Melissa officianlis* displays anti-cancer potential.

soil, the year harvested, and temperature, influence the number of ingredients in a plant extract.[20]

When it comes to different extracts of *M. officinalis*, methanolic extract shows more neurological activities than aqueous extracts. On the other hand, aqueous and ethanolic extracts of *M. officinalis* isolated from the aerial part are traditional medicine to treat colds, fevers, toothaches, nervous tension, and mild insomnia.[18,21]

## Anti-cancer Effect of *M. officinalis*

Studies have proven that *M. officinalis* contains agents that show anti-cancer activity (Table 1). Hydroalcoholic extract of lemon balm possesses a strong potential to inhibit the proliferation of various cancerous tissues in a dose-dependent manner.[6] Phenolic compounds of *M. officinalis* exhibit a wide range of anti-cancer activities, including antiproliferation, antiangiogenic, antimigratory, antioxidant, and antiapoptosis on cancer cells (Figure 1). As one of the phenolic components, Ursolic acid has remarkable toxicity on various tumors, including breast carcinoma, melanoma, hepatoma, prostate carcinoma, and acute myelogenous leukemia.[24] *Melissa* extracts as a phytochemically active plant can also be applied in phytotherapy, reducing cancer occurrence, particularly in colon tumors, since the concentration of orally consumed plant extracts is high in the gut and microgram per

**Table 1**. Examples of different types of *Melissa officinalis* extracts investigated on cancer cells.

| Mechanism | Type of extract/ingredient | Cancer cell | Location | References |
|---|---|---|---|---|
| Dox-induced cardiotoxicity | Ethanol | MCF-7 | Syria | 6 |
| Antiangiogenic | Aqueous | A549<br>MCF-7<br>PC3 | Iran | 25 |
| Antiproliferation | Ethanolic<br>Aqueous | HCT-116 | Spain | 12 |
| Antiapoptotic | Methanol | K562 | Iran | 16 |
| Antiangiogenesis | Ethanol | MCF-7<br>MDA-MB-231<br>MCF-10A | Romania | 26 |
| Antiproliferation | Hydroalcoholic<br>PBS | SKOV3<br>MCF-7<br>PC-3<br>A549 | Iran | 21 |
| Antiapoptotic | Aqueous | MCF-7<br>MDA-MB-468<br>MDA-MB-231 | Turkey | 17 |
| Antioxidant | Essential oil | HL-60<br>K562<br>A549<br>Caco-2<br>MCF-7<br>B16F10 | Brazil | 15 |
| Antiapoptotic | Essential oil | A172<br>U87 | Brazil | 11 |
| Antioxidant<br>Antiapoptotic | Hydroethanolic | HT-29 | Germany | 23 |

milliliter, it is enough to show anti-cancer effect.[23]

### Antiproliferative Effect of *M. officinalis*

It is currently shown that herb-based products like plant extracts as a natural resource of chemical compounds, particularly phenols with high antioxidant capacitary, display antiproliferative effect on a wide range of cancer cells, including PC-3, NCI-H82, MCF-7, Hep-3B, and K-562 as a prostate, small cell lung, breast, liver, and chronic myeloid leukemia cancer cells, respectively.[17] In a study, Jahanban-Esfahlan *et al.*[16] evaluated the anti-cancer efficacy of *M. officinalis* in various Human cancer cell lines. They synthesized a hydroalcoholic extract of *M. officinalis* and then measured total phenolic and flavonoid content. The MTT assay results showed that *M. officinalis* reduced cell viability below 33% in the lowest concentration in PC-3, A549, SKOV3, and MCF-7 cancer cells. They concluded that the hydroalcoholic extract of *M. officinalis* could inhibit the proliferation of different tumor cells in an optimal biological dose.

Furthermore, *M. officinalis* exhibited a tumor type-specific cytotoxicity in which hormone-dependent cancers were more sensitive to the anti-cancer properties of this extract. Encalada *et al.*[13] investigated the cytotoxicity of aqueous and ethanolic extract of *M. officinalis* via cell viability assay on human colon cancer cells. Based on the achieved results, the ethanolic extract of *M. officinalis* caused the highest cell growth inhibition with the lowest dose tested (5 µg/ml) after 72 h, leading to reduced cell proliferation near to 40% in HCT-116 cancer cells. They showed that the antiproliferative activity and antioxidant effect of *M. officinalis* was associated with the total phenolic and flavonoid content, especially R.A, considered an antiproliferative compound in this study. They suggested that R.A can be a useful anti-cancer compound combined with conventional anti-cancer drugs to treat human colon cancer.[13]

### Antiangiogenic Effect of *M. officinalis*

Angiogenesis is a biological process in which new blood vessels develop from pre-existing ones. As it is known, angiogenesis plays a crucial role in deregulated and pathological problems in cancer development.[12] The tumor tissue can get nutrients from new blood vessels more efficiently, resulting in a cancer metastasis facility. Diabetic retinopathy and nephropathy of some diabetic manifestations are two significant pathogenetically fetal problems caused by abnormalities in angiogenesis.[18,27] *M. officinalis,* as traditional medicine, prevents neovascularization (CNV) development, which is laser-induced choroidal via MMP-9 and vascular endothelial growth factor (VEGF) inhibition. Besides, mRNA levels

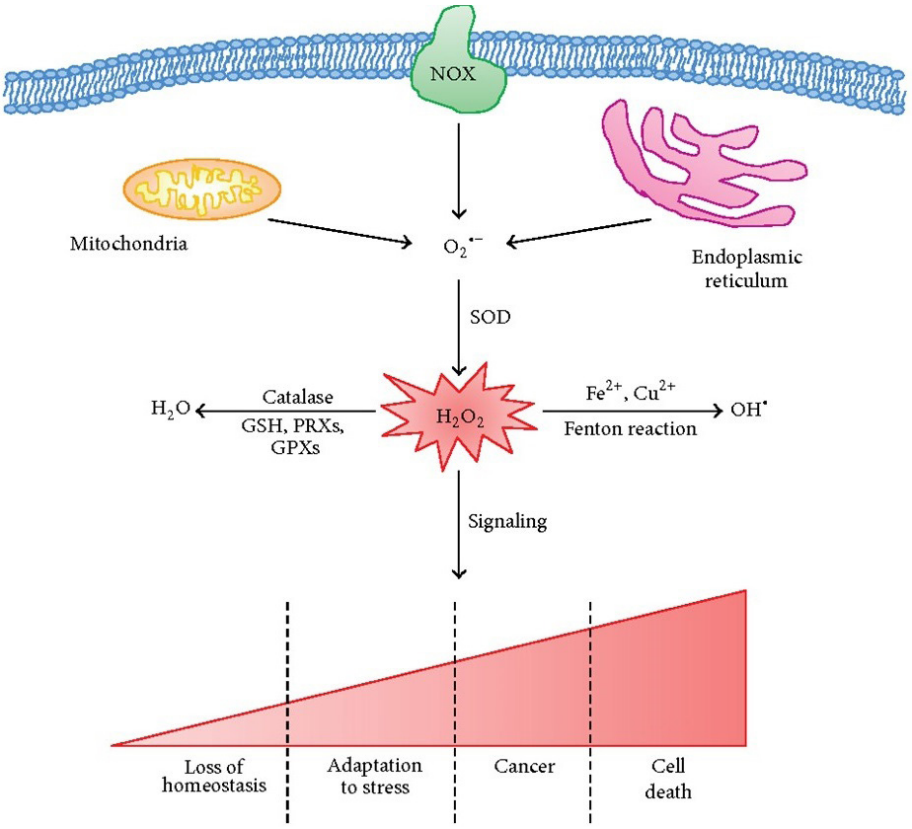


**Figure 2.** Schematic illustration of ROS production in cancer cells. Mitochondria, NAPH oxidase (NOX), and endoplasmic reticulum are responsible for intracellular ROS generation. $O_2$ as a main form of ROS, converts to hydrogen peroxide ($H_2O_2$), which is carried out by superoxide dismutase (SOD). The amount of ROS production defines cell fate. Low and intermediate levels of the peroxide stimulate loss of cell homeostasis and increased adaptation to stress leading to neoplastic transformation, while high levels induce cell death. Republished with permission from ref. 30.

of angiogenic factors including fibroblast growth factor-2 (FGF-2), MMPs (MMP-9 and MMP-2), and VEGF-A, -B, -C, -D show a reduction upon treatment with *M. officinalis* known as an antiangiogenic formulation. At the same time, the mRNA level of angiogenic inhibitors experienced an increase in distinguished cells.[28] p53 is another crucial molecule that is responsible for angiogenesis, apoptosis, and cell cycle control. Mutation in p53 leads to overexpression of Bcl-2 protein in various types of tumors. Cellular proliferation, differentiation, and angiogenesis are several biological processes regulating the PI3K/Akt pathway.[16]

R.A can inhibit several angiogenic stages, such as migration, proliferation, tube formation, and adhesion in human endothelial cells.[18,29] Jahanban-Esfahlan *et al.*[16] used *M. officinalis* as an anti-cancer agent to investigate the molecular mechanism that is related to the cytotoxicity effect of this extract. They measured the expression level of various molecules with cancer treatment potential in MCF-7, A549, and PC3 cancer cells. The results showed that *M. officinalis* demonstrated high angiogenic activity related to the high antitelomerase activity in the mentioned cancer cells. They showed that hydroalcoholic extract of *M. officinalis* inhibited the expression of Bcl2, Her2, VEGF-A, and hTERT as a prominent oncogene in PC3 cancer cells. Moreover, in MCF-7 and A549 cancer cells, *M. officinalis* indicated its anti-cancer effect via the downregulation of VEGF-A and hTERT molecules. They noticed that the therapeutic potential of *M. officinalis* on human breast, lung, and prostate cancer cells could be accounted for expression modulation of VEGF-A and hTERT genes.[16]

## Antioxidant Effect of *M. officinalis*

Nitrogen species and reactive oxygen (RNS and ROS) are two types of highly reactive molecules produced via normal metabolic processes and external factors in all organisms. Oxidative stress (O.S.) can occur through the inactivation of physiological antioxidant defense in response to excessive production of RNS and ROS.[17] In cancer treatment, anti-cancer drugs produce cardiomyocytes, and plasma membrane damage mediated oxidative damage and led to cancer cell death by apoptosis (Figure 2).[30] Cardiotoxicity caused by conventional chemotherapeutic medicines cannot be overcome even using synthetic agents like antioxidants and metal chelators. In stark contrast, medical plants successfully eliminate cardiotoxicity associated with cancer treatment. Therefore, it is sensible to discover more about plant derived-natural compounds to eradicate cardiotoxicity and improve treatment efficacy.[10]

On the other hand, plant-based components with high antioxidant activity exhibit an antitumor effect via developing reactive oxygen species (ROS) on various cancer cell lines. Lemon balm shows intense antioxidant effects, which is 10 folds higher than the antioxidant effects of vitamin C and vitamin B.[22,31] Chemical components responsible for forming reactive oxygen species are caffeic acid, rosmarinic acid, ferulic acid, syringic acid, and protocatechuic acid. However, it is not a reason to consider just single compounds to explain the effects of the whole plant based on the complexity of plant extracts.[23] In a study, Hamza *et al.*[6] investigated the ameliorating impact of *M. officinalis* in response to cardiotoxicity, which is mediated oxidative stress caused by DOX therapy in cancer disease. *In vivo* study indicated that oral administration of *M. officinalis* protected heart tissue of rats from histological changes, which is due to the DOX-induced leakage of cardiac enzymes. The decrease in lipid peroxidation, depletion in total oxidant capacity, and protein oxidation are dominant marks of amelioration of DOX-induced oxidative through *M. officinalis*.[6]

Moreover, pretreatment with *M. officinalis* significantly impacts nuclear factor kappa-B, cyclooxygenase-2, tumor necrosis factor-alpha, and myeloperoxidase activity inhibiting inflammatory regarding DOX therapy. They concluded that *M. officinalis* with DOX potentiated the therapeutic efficiency of DOX and alleviated its cardiotoxicity in human breast cancer therapy. The anti-cancer effect *of M. officinalis* was due to ROS production on specific cancer cell lines; however, independent mechanisms induce its free radical scavenging activity.[6,32] In another study, Weidner *et al.*[23] investigated hydroethanolic extract of *M. officinalis* to show prooxidative rather than antioxidative effect. The results showed that apoptosis induction and cytotoxicity of the *M. officinalis* increased upon ROS formation in colon cancer cells, which was as good as potent antioxidants such as glutathione *N*-acetyl cysteine. They showed that phenols and flavonoids provide a balance between oxidation and antioxidation production in cancer cells. In other words, redox-active molecules play a role in the cellular redox state and scavenge free radicals and keep constant antioxidant enzyme levels. As a prooxidant, it substantially affects metal ions, leading to ROS production like hydrogen peroxide and hydroxyl radicals. Indeed, the pro/antioxidants role of *M. officinalis* can be attributed to the dose of natural product, micro oxygen environment, the concentration of metal ions, and the presence of other pro/antioxidants.[23] de Sousa *et al.*[33] assessed the biological activities of essential oil of *M. officinalis* on MCF-7, Caco-2, K562, A549, and HL-60 cancer cells. They showed that essential oil could reduce 1-diphenyl-2-picrylhydrazyl (DPPH), which is a sign of the antioxidant activity of this compound. As one of the essential oil components, geraniol inhibited the viability of human colon, pancreatic, skin, and hepatic cancer cells.

Taken together, ROS production regulates hemostasis and cell signaling, although an imbalance in its concentration causes various disorders, including cancer. A high ROS generation in cancer cells due to accelerating metabolism than normal cells sensitize them to oxidative stress-induced cell death. Hence, increasing the ROS level can be considered a suitable strategy to surpass cancer cell growth.[17] This study revealed that *M. officinalis* extract could eliminate colon cancer cells using ROS- induction mechanism and change in ROS balance based on rosmarinic

acid, caffeic acid, and a ferulic acid component.[33-35]

### Antimigratory effect of *M. officinalis*

Cell migration as a complex process is essential for regeneration, tissue repair, and physiological development. However, metastasis, which is the main reason for morbidity and mortality in cancer cells, arises from migration.[36] It has been shown that the migration of cancer cells is not carried out randomly, i.e., various types of cancer cells take different destinations. Soil and seed theory explain this property of cancer cells vividly. Based on this theory, different organs have specific and optimized conditions for the growth of definite cancer cells. In contrast, homing theory explains that different organs arrest or absorb particular cancer cells via chemotactic factors.[37]

Migration allows neoplastic cells to change their position and enter lymphatic and blood vessels to circulate through blood flow and then undergo metastatic growth in distant organs. Embryonic morphogenesis, immune-cell trafficking, and wound healing are physiological processes that cancer cells use to spread through the tissues. Cancer cells undergo a modification in shape and stiffness to interact with the structure surrounding tissue.[37,38] The first step in cell migration is polarization and elongation in cancer cells, forming a pseudopod from the extended edge of cells to attach to the ECM substrate.[38,39] Cell extensions are the main and requested part of cell migration in cancer cells caused by either growth factors and chemokines induction or spontaneously. When it comes to *M. officinalis*, several studies investigated the metastatic feature of this herb using the scratch assay.[38] In a study, Moaca *et al.*[19] used leaves and stems extracts of *M. officinalis* to assess the wound healing effect. The result showed that stems extract of *M. officinalis* could display the best antimigratory effect even in low concentration compared to leaves extract in the same dose after 3h of stimulation on HaCat cells. They showed that after 24h of treatment, the cancer cells onset detaching, rounding, and change in shape and morphology. Ethanolic extract of *M. officinalis* indicated the same antimigratory effect in the HaCat cells. Following previous studies, the authors concluded that rosmarinic acid from *M. officinalis* extracts accounts for the antimigratory effect of cancer cells.[38,40]

### *M. officinalis* and Cell Cycle Arrest Activity

P53 independent and dependent pathways are two different mechanisms controlling cell cycle arrest in cancer cells. G2M arresting stems from up-regulating p21 expression and inactivation of cdk1 involving topoisomerase II inhibition in p53 null cells.[41,42] The arrest of G0/G1 can occur due to activation of the p21-Rb pathway and inactivation of Rb-phosphorylation simultaneously.[1,43] In a study, Stanojkovic *et al.*[24] investigated the cell cycle arrest effect of *M. officinalis* on MDA-MB-361, MDA-MB-453, and HeLa cancer cells. Provided aqueous-ethanol extracts of *M. officinalis* showed significant cytotoxicity on Human breast and cervical cancer cells. Content of total phenol and tannin compounds like rosmarinic acid and ursolic acid was in the same quantity in both extracts. They concluded that Extract 1 and Extract 2 are rich sources of bioactive compounds hindering cell cycle development in the G1 phase, resulting in apoptosis triggering via DNA fragmentation.

However, each extract illustrated a different potential in the percentage of the sub-G1 population when exposed to breast and cervical cancer cells. These data showed that G1 phase cell cycle arrest involved G1 checkpoint, which is carried out via p53 as this molecule plays a vital role in cell cycle arrest, and its mutation leads to defection in the checkpoint. Besides, reducing cancer cells in the G2=M phase showed that both Extract 1 and Extract 2 had a dose-dependent antiapoptotic effect. They concluded that both extracts induced apoptosis in a dose-dependent manner with a rise in the sub-G1 population in breast and cervical cancer cells.[24] In another study, Jahanban-Esfahlan *et al.*[16] assessed the tumor inhibitory effect of *M. officinalis* extract, including aqueous, ethanolic, hydroethanolic, methanolic, and hydroethanolic on MCF-7, NCI-H460, and AGS cancer cell lines. The data revealed that ethanolic extract was the most effective extract, and NCI-H460 was the most affected cell. However, the aqueous extract did not show a significant change in the viability of cancer cells.

Regarding the effect of ethanolic extract of *M. officinalis* on cell cycle arrest, the nuclear DNA content of cancer cells was analyzed. It showed a remarkable reduction in the percentage of cancer cells in the G2/M phase of the cell cycle in a dose above $IC_{50}$ concentration. Thus, they concluded that *M. officinalis* has a dose-dependent effect on the cell cycle profile of NCI-H460 lung cancer cells. Measuring the expression level of the protein involved in the cell cycle and apoptosis showed that p53 expression amount increased when treated with $IC_{50}$ and $IC_{75}$ concentrations of ethanolic extract of *M. officinalis*. This result proved that the change in the expression level of p53 protein could be responsible for cell cycle phase alteration and increase in apoptosis.[16]

### *M. officinalis* Effect on Apoptosis Mechanism

Cancer is the most prominent disease occurred via abnormalities in the cell death process. Hence, regulating the death process and determining molecules responsible for apoptosis is vital to prevent and manage these types of human diseases.[6] Apoptosis is a potential mechanism for cell death in cancer research, controlling cell-suiciding programs genetically and is carried out via extrinsic and intrinsic pathways.[12] In the extrinsic pathway, stimulation of the death receptors plays a crucial role in forming the death-inducing signaling complex (DISC) in the plasma membrane. Therefore, the DISC complex activates caspases 8 and 9. As a critical linker of extinct and intrinsic apoptosis pathway, stimulating procaspase-3 activation or cleaving Bid to (t) Bid directly leads to promoting the induction of mitochondrial outer membrane permeabilization (MOMP).[41,44]

Regarding the intrinsic pathways, Bax, as a pro-

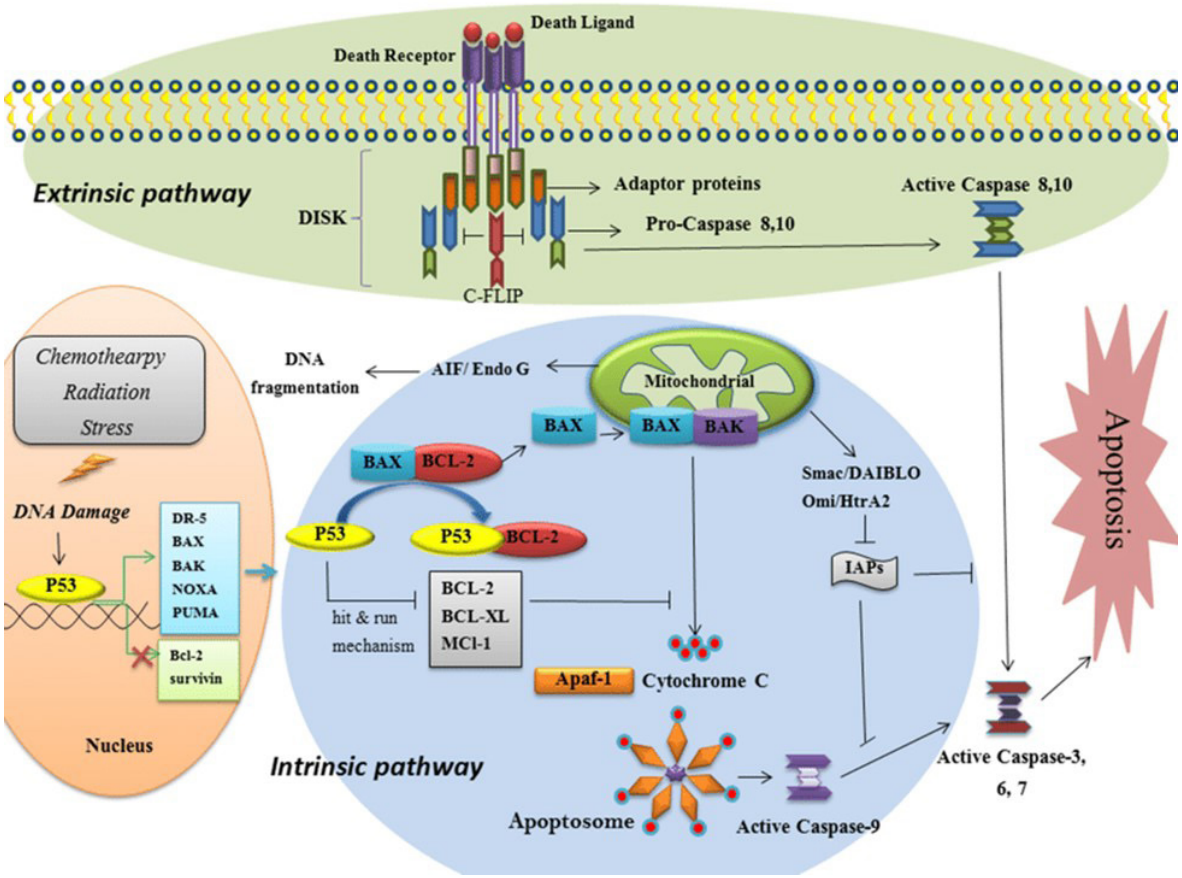


**Figure 3.** Schematic diagram of the intrinsic, extrinsic pathways of apoptosis. Both apoptotic pathways lead to the same function, which is cell death. The initiator of the extrinsic pathway is the attachment of extracellular ligands to the extracellular domain of transmembrane receptors. However, several intracellular stimuli are the initiator of mitochondrial apoptosis. Republished with permission from ref. 45.

apoptotic protein and primary activator of MOMP formation, accelerates and triggers apoptosis. Bcl-2 is an antiapoptotic molecule that disrupts the MOMP formation and consequently surpasses apoptosis progression.[28,41] *M. officinalis* upregulate proteins triggering an apoptosis mechanism consisting of cytochrome C, p53, and Bax. These proteins are linked to mitochondria and regulate apoptosis via mitochondrion permeability modulation. Bcl-2 protein can be surpassed via p53 as a tumor suppressor protein (Figure 3).[45] Subsequently, cytochrome C release leads to promoting caspase cascade.[6]

Also, the free radical scavenging capacity of *M. officinalis* led to a significant decrease in Bax and caspase 3 protein expression levels. Caspase 7, a critical protein in triggering apoptosis, is activated in extrinsic and intrinsic pathways. Many substrates are cleaved using mature caspases-3 and -7 given rise to biochemical and morphological indications in programmed cell death.[10] In a study, Ebrahimnezhad Ebrahimnezhad Darzi *et al.*[41] examined the cytotoxicity and apoptosis induction capability of *M. officinalis* on K562 and Jurkat cancer cells. Various fractions of *M. officinalis* displayed different effects on leukemia cell lines. They showed that both n-hexane and dichloromethane fractions induced apoptosis and caused accumulation of cancer cells in the sub-G1 phase and arrested in G2/M phase and G0/G1 18 h after treatment in a time and dose-dependent manner. As K562 and Jurkat cancer cells are p53 nulls, they concluded that the cell cycle arrest and apoptosis induction was not related to the p53 signaling pathway.

Moreover, the dichloromethane fraction increased the expression of Fas and Bax proteins and led to the activation of both extrinsic and intrinsic apoptosis pathways altogether. In other words, the apoptosis induction effect of *M. officinalis* can be attributed to the dichloromethane fraction in this study.[18] A varied range of components from non-polar to polar are extracted via dichloromethane, which could interact with the cell membrane and pass the cancer cells.[41] In another study, Saraydin and her colleagues investigated the antiproliferation effect of *M. officinalis,* indigenous of Turkey, on breast cancer cells. The obtained results indicated that *M. officinalis* had a cytotoxicity effect on all MCF-7, MDA-MB-468, and MDA-MB-231 cancer cells. Besides, the treatment of the cancer cells with *M. officinalis* increased the number of Annexin-positive cells. The *in vivo* study demonstrated that rats treated with *M. officinalis* had an increased caspase 7 expression and Tunnel positive cells compared to control groups. The tumor volume decreased to 40% in treated groups compared to untreated rats.[13]

On the other hand, several morphological changes such as DNA fragmentation and the breakdown of the cell into apoptotic bodies characterize the apoptotic process. Nuclear

DNA breakdown into nucleosome-sized fragments, called DNA fragmentation, is an obvious biochemical signature of apoptosis and represents the most intense damage to cancer cells.[46] Nuclear shrinkage, apoptotic body formation, and chromatin condensation are other morphological changes of programmed cell death. The mechanism developing apoptotic DNA fragmentation and, consequently, nuclear changes is caspase-activated DNAse (CAD), also known as a DNA fragmentation factor (DFF).[13,47] ICAD is a natural inhibitor of CAD, which inactivated it in nonapoptotic cells via binding to this molecule. Activated caspase 3, 7 cleave DFF resulted in chromosomal DNA fragmentation by generating double-stranded breaks.

The additional factors that stimulate endonuclease activity *in vitro* are histone H, high mobility group proteins, and topoisomerase II, facilitating DFF40-mediated DNA fragmentation. Besides, chromatin condensation is induced via DFF molecules *in vitro* in DFF45-deficient cells compared to normal cells mediated via caspase-9 – caspase-3 pathway during apoptosis activation.[48] The mitochondrial apoptotic endonuclease G (EndoG) is another nuclease that plays a crucial role in the complete removal of DNA during apoptosis. Following activation of apoptosis, EndoG release, which is similar to cytochrome *C* release, into the mitochondrial intermembrane space, and its traveling to the nucleus lead to the production of nucleosome DNA fragmentation.[47,49] In other words, EndoG is a molecule that cleaves chromosomal DNA in DFF45-deficient cells in response to specific apoptotic stimuli in a caspase-independent way.[50] Although DFF has intense endonuclease activity and breaks double-stranded DNA, and can generate single-stranded DNA breaks. Apoptosis-inducing factor (AIF) as a flavoprotein is released from the mitochondrial intermembrane space in response to apoptosis activation and migrates to the nucleus, brought about partial chromatin condensation and DNA degradation. Similar to EndoG, caspase activation is not necessary to mediate DNA degradation.[51,52]

## Conclusion

The strategy of herbal plants contains a mixture of active components that are traditionally used as a combination therapy for increased synergistic effects in treating diseases like cancer. Many medicinal plants in nature have been studying for their anti-cancer properties to discover cost-effective and safe cancer drugs. Given *M. officinalis*, it is one of the most used medical-based plants, especially herb tea, to cure different diseases such as cancer. The current studies proved its antitumor efficacy on several types of cancer, especially lung, breast, prostate, colon, and cervical cancers. The anti-cancer effect of the active component of *M. officinalis* extracts is through various biological mechanisms such as antiproliferation, antiangiogenics, antiapoptotic, cell cycle arrest, and antioxidant. This review explained different active ingredients and molecules, playing a crucial role in the mentioned processes. According to this review, *M. officinalis* is a potent anti-cancer agent that can be used to treat cancer cells. American Food and Drug Administration (FDA) has recognized *M. officinalis* extract as a safe herb, which can be used as a tea. Although further *in vivo* and clinical studies are needed to assess the practical health effect of *Melissa* extracts in cancer treatment. Besides, mechanisms of actions and pharmacokinetics of various active compounds of *M. officinalis* extract should be elucidated to reach a realistic dosage in cancer treatment. Finally, investigation of the Phytomedical and nutraceutical properties of *M. officinalis* may provide promising approaches to eradicate cancer disease.

## Author Contributions

PF: Writing original draft, MA: Conceptualization, MG: Writing original draft, JEND: Conceptualization, Supervision and Writing - review & editing. All authors read and approved the final manuscript.

## Conflict of Interest

The authors report no conflicts of interest.

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