

## REVIEWS

# ESSENTIAL OILS AS MULTI-TARGET BIOACTIVE SYSTEMS: A REVIEW OF CHEMICAL DIVERSITY, GC-MS PROFILING AND ANTIMICROBIAL, ANTIOXIDANT, AND ANTITUMOR POTENTIAL

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## ABSTRACT

Essential oils are complex multicomponent mixtures of volatile plant-derived secondary metabolites that have attracted increasing scientific interest due to their broad spectrum of biological activities. Owing to their chemical diversity and multitarget mechanisms of action, essential oils represent promising bioactive systems with antimicrobial, antioxidant, and antitumor potential. The present review provides a comprehensive analysis of current literature data on the chemical composition of essential oils, with particular emphasis on GC-MS profiling as a prerequisite for reliable interpretation of biological activity. Variations in chemotype, botanical origin, geographical factors, and extraction methods are discussed as key determinants of bioactivity. The review summarizes experimental evidence demonstrating strong antimicrobial effects of essential oils, especially those rich in phenolic monoterpenes such as carvacrol, thymol, and eugenol, including activity against multidrug-resistant pathogens and biofilm-forming microorganisms. Particular attention is given to synergistic interactions between essential oils and conventional antibiotics, which result in reduced minimum inhibitory concentrations and enhanced antimicrobial efficacy. In addition, antioxidant properties of essential oils are critically evaluated using common *in vitro* assays (DPPH, ABTS, FRAP), highlighting the contribution of synergistic interactions among major and minor constituents. Furthermore, accumulating *in vitro* data on the antitumor activity of essential oils are reviewed, including mechanisms such as apoptosis induction, cell cycle arrest, and oxidative stress modulation. Overall, essential oils emerge as multifunctional bioactive systems with significant potential as natural antimicrobial adjuvants, antioxidants, and complementary agents in anticancer research. However, their clinical translation is currently limited by compositional variability, insufficient standardization, and the predominance of *in vitro* data. The review emphasizes the need for standardized analytical protocols, comprehensive toxicological evaluation, and well-designed *in vivo* and clinical studies to fully assess the therapeutic applicability and safety of essential oils.

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Received: September 29, 2025

Accepted: December 6, 2025



## INTRODUCTION

Essential oils are complex multicomponent mixtures of volatile secondary metabolites of plant origin, synthesized in various plant organs. For centuries, they have been used in traditional medicine, perfumery, pharmacy, and the food industry, while in recent decades, scientific interest in these natural products has increased substantially due to their broad spectrum of biological activities, including antimicrobial, antioxidant, and antitumor effects (1–3). Essential oils differ from conventional synthetic drugs in that their biological activity is not attributed to a single active compound, but rather to the synergistic interactions of multiple constituents. These complex mixtures, composed mainly of monoterpenes, sesquiterpenes, and their oxygenated derivatives, act on multiple biological targets, resulting in a broad spectrum of effects (4,5). This complex mode of action makes essential oils particularly promising in the context of antibiotic resistance, oxidative stress, and the complex nature of chronic and malignant diseases (6–8). Essential oils exhibit broad-spectrum activity and multitarget mechanisms capable of enhancing the efficacy of existing antibiotics and contributing to the mitigation of antimicrobial resistance. Consequently, they have attracted increasing attention as natural agents against priority pathogens and multidrug-resistant strains (9). At the same time, their therapeutic potential is being investigated across a wide range of conditions, from infectious to inflammatory and oncological diseases. Nevertheless, further standardization and robust clinical evidence are required, as factors such as variable chemical composition, dosage, and potential toxicity remain critical considerations (10). Gas chromatography coupled with mass spectrometry (GC–MS) is widely recognized as a key analytical technique for the qualitative and quantitative characterization of essential oil composition (11–13). This technique enables reliable identification of individual constituents, as well as detailed assessment of compositional variability related to botanical species, geographical origin, climatic conditions, developmental stage of the plant, and extraction method. The availability of standardized GC–MS profiles provides a sound basis for correlating chemical composition with observed biological activity, which is of particular importance given the complex nature of essential oils

and the potential for synergistic interactions among their components.

## AIM

The aim of the present review is to summarize and critically analyze current literature data concerning the chemical diversity of essential oils, their GC–MS profiles, and the relationship between chemical composition and antimicrobial, antioxidant, and antitumor activities. Emphasis is placed on their potential as pharmaceutically relevant multicomponent bioactive systems, their synergistic interactions with conventional therapies, prospects for future applications, as well as existing limitations and the need for further research.

## MATERIALS AND METHODS

The present work represents a thematic literature review based on systematic searching and analysis of scientific publications. Sources were selected from the Scopus, Web of Science, PubMed, and Google Scholar databases using combinations of the following keywords: *essential oils*, *GC–MS analysis*, *chemical composition*, *antimicrobial*, *antioxidant*, *anticancer*, *biofilm*, and *synergy*. Original research articles and review papers published predominantly between 2000 and 2025 were included, with priority given to more recent studies, particularly those from 2023–2025. Inclusion criteria comprised clear identification of chemical composition (primarily via GC–MS) and reliable characterization of biological activity (*in vitro* and/or *in vivo*), with particular attention paid to studies addressing synergistic effects, antibiofilm activity, and mechanisms of antitumor action. Data processing involved comparative analysis of chemical profiles of different essential oils and chemotypes, as well as evaluation of antimicrobial activity parameters (minimum inhibitory concentrations and minimum bactericidal concentrations), antioxidant activity, and cytotoxic effects on tumor cell lines, including mechanisms related to induced cell death.

## RESULTS

### *Chemical Diversity of Essential Oils and GC–MS Profiling*

Analysis of the available literature data demonstrates that essential oils are chemically highly

complex systems, containing from 20 to more than 100 identifiable components (in some cases even exceeding 200). The major classes of compounds include monoterpenes, sesquiterpenes, and phenylpropanoids, with their relative proportions varying substantially among different plant species, chemotypes, and even populations from distinct geographical origins. For example, the essential oil of *Origanum vul-*

variations in the carvacrol/thymol ratio result in significant differences in effectiveness against *Staphylococcus aureus* and *Escherichia coli*. Therefore, interpretation of the biological activity of an essential oil should always be accompanied by comprehensive GC–MS characterization of its composition in order to account for the effects of chemical variability.

*Table 1. GC–MS profiling and chemotypic variability of essential oils.*

Plant Species	Major Constituents	Chemotype	References
<i>Origanum vulgare</i> L.	Carvacrol	Phenolic	(20)
<i>Thymus vulgaris</i> L.	Thymol	Thymol-type	(21)
<i>Thymus vulgaris</i> L.	Linalool	Linalool-type	(21)
<i>Rosmarinus officinalis</i> L.	1,8-Cineol	Cineol-type	(22)
<i>Rosmarinus officinalis</i> L.	Camphor	Camphor-type	(22)
<i>Lavandula angustifolia</i> Mill.	Linalool	Linalool-type	(23)
<i>Satureja montana</i> L.	Carvacrol	Phenolic	(24)
<i>Ocimum basilicum</i> L.	Linalool	Linalool-rich chemotype	(25)
<i>Salvia officinalis</i> L.	Thujone	Ketone-type	(26)
<i>Zataria multiflora</i> Boiss.	Thymol	Phenolic	(27)

*gare* may be dominated by phenolic monoterpenes such as carvacrol or thymol, depending on the chemotype, whereas several chemotypes of *Thymus vulgaris* have been described, including thymol-, linalool-, and other chemotypes with markedly different compositions (14–17). GC–MS profiling enables not only the identification of major constituents but also the detection of numerous minor compounds that may contribute to the overall biological activity of essential oils (18,19). The absence of a detailed chemical profile may lead to contradictory and poorly reproducible results when comparing the bioactivity of essential oils of apparently identical origin.

Table 1 presents examples of GC–MS profiling and chemotypic variability in several widely studied essential oils. These data illustrate how the same plant species can produce different chemotypes dominated by distinct components, which directly influences the observed biological activity. For instance, rosemary oil (*Rosmarinus officinalis*) with 1,8-cineole as the main constituent (the so-called cineole chemotype) exhibits weaker antimicrobial activity compared with camphor- or  $\alpha$ -pinene-rich chemotypes. A similar relationship is observed for oregano, where

**Note:** The table summarizes the dominant components and the corresponding chemotypes of selected essential oils. Various sources (20–27) report compositional variability, for example oregano (phenolic chemotype rich in carvacrol), thyme (thymol and linalool chemotypes), rosemary (cineole vs. camphor chemotypes), among others. These chemical differences correlate with variations in the antimicrobial and antioxidant activities of the oils.

#### **Antimicrobial Activity**

Antimicrobial activity is the most extensively documented biological function of essential oils. Data from *in vitro* studies (MIC—minimum inhibitory concentration, MBC—minimum bactericidal concentration, etc.) indicate that essential oils rich in phenolic monoterpenes—such as carvacrol, thymol, and eugenol—exhibit particularly strong activity against Gram-positive bacteria (2,28). The increased susceptibility of Gram-positive pathogens (e.g., *Staphylococcus aureus* and *Listeria monocytogenes*) is attributed to the simpler structure of their cell wall and the absence of an outer membrane, which facilitates interactions between lipophilic oil

components and membrane lipids (2,29,30). In contrast, the complex outer membrane of Gram-negative bacteria limits the penetration of hydrophobic molecules, rendering them generally less susceptible. Nevertheless, numerous studies have demonstrated that essential oils with high contents of carvacrol and thymol (e.g., oregano and thyme oils) are capable of effectively disrupting membrane permeability and integrity even in Gram-negative bacteria, leading to leakage of ions and intracellular metabolites (2,31). These findings support the multimodal mechanism of action of essential oils, which involves simultaneous membrane damage, enzyme and protein denaturation, and metabolic disruption, ultimately result-

ing in rapid bactericidal effects. linalool) show activity even against resistant strains such as MRSA (methicillin-resistant *Staphylococcus aureus*), although higher MIC values are generally required compared to phenolic-rich oils (35,36). *Melaleuca alternifolia* (tea tree oil, terpinen-4-ol) has been successfully applied against skin infections and demonstrates antimicrobial activity against *Staphylococcus aureus* and dermatophytes (37).

Collectively, these data confirm that essential oils can act as broad-spectrum antimicrobial agents, including activity against difficult-to-treat pathogens, although their efficacy strongly depends on oil composition and concentration.

Table 2. Antimicrobial activity of essential oils.

Essential Oil	Major Constituents	Microorganism	Method	References
<i>Thymus vulgaris</i>	Thymol	<i>S. aureus</i>	MIC	(20,36)
<i>Origanum vulgare</i>	Carvacrol	<i>E. coli</i>	MIC	(31)
<i>Satureja montana</i>	Carvacrol	<i>S. aureus</i>	MIC	(24)
<i>Cinnamomum verum</i>	Cinnamaldehyde	<i>E. coli</i>	MIC	(32)
<i>Syzygium aromaticum</i>	Eugenol	<i>S. aureus</i>	MIC	(33)
<i>Zataria multiflora</i>	Thymol	<i>P. aeruginosa</i>	MIC	(34)
<i>Ocimum basilicum</i>	Linalool	MRSA	MIC	(35)
<i>Lavandula angustifolia</i>	Linalool	<i>S. aureus</i>	MIC	(36)
<i>Melaleuca alternifolia</i>	Terpinen-4-ol	<i>S. aureus</i>	MIC	(37)
<i>Cymbopogon citratus</i>	Citral	<i>S. aureus</i>	MIC	(38)

ing in rapid bactericidal effects.

Table 2 summarizes representative data on the antimicrobial activity of various essential oils against selected pathogenic microorganisms. The main active components, tested microorganisms, and evaluation methods are presented. Notably, *Thymus vulgaris* (thyme) and *Satureja montana* (savory), both rich in thymol and carvacrol, exhibit low MIC values, indicating strong activity against *Staphylococcus aureus*, a clinically relevant Gram-positive pathogen (24,30). *Syzygium aromaticum* (clove oil), characterized by a high eugenol content, also demonstrates potent antimicrobial effects (33). *Cinnamomum verum* (cinnamon, containing cinnamaldehyde) and *Cymbopogon citratus* (lemongrass, rich in citral) are effective against *Escherichia coli* and *Staphylococcus aureus*, respectively (32,38). *Ocimum basilicum* (basil, linalool) and *Lavandula angustifolia* (lavender,

**Note:** MIC (minimum inhibitory concentration) is defined as the lowest concentration (expressed in mg/mL or µg/mL) of an essential oil that inhibits the visible growth of a given microorganism. In many cases, such as *Thymus vulgaris*, *Origanum vulgare*, and *Satureja montana* essential oils, MIC values against *Staphylococcus aureus* are reported to be low, indicating strong antibacterial activity (2, 24). For resistant strains, including MRSA, higher concentrations are generally required; however, combination with conventional antibiotics may result in synergistic effects and a reduction in MIC values (2,30). Oral pathogens, such as *Streptococcus mutans* and related species, are particularly sensitive to eugenol-rich clove (*Syzygium aromaticum*) essential oil (33).

### Antibiofilm Activity and Synergy with Antibiotics

In recent years, increasing attention has been paid to the ability of essential oils to inhibit microbial biofilms. Biofilms are complex structures in which microorganisms are embedded within an extracellular polymeric matrix, significantly increasing their resistance to antibiotics and disinfectants. Available literature indicates that certain essential oils are capable of both inhibiting early stages of adhesion and biofilm formation, as well as disrupting already established biofilm structures (18, 39).

For example, phenolic components such as thymol, carvacrol, and eugenol can disrupt the integrity of the biofilm matrix and enhance antibiotic penetration. The combination of essential oils with conventional antibiotics frequently results in synergistic effects, manifested as a significant reduction in MIC

Staphylococcus aureus, resulting in a significant reduction in MIC values and indicating synergistic interactions (18). Similar effects have been reported for combinations of essential oils rich in phenolic compounds, such as oregano and thyme, with conventional antibiotics (40,41). Other essential oils (e.g., cinnamon, tea tree, and basil) have also demonstrated additive or synergistic effects when combined with different classes of antibiotics, including fluoroquinolones and other antimicrobial agents (31,39).

Examples of such combinations are summarized in Table 3, highlighting the potential of essential oils to enhance antibiotic activity and effectively target biofilm-associated infections. These findings support the concept of using essential oils primarily as adjuvants rather than as standalone therapeutic agents, particularly in the context of multidrug-resistant infections.

Table 3. Synergy between essential oils and antibiotic.

Essential Oil	Antibiotic	Model (Organism/system)	Effect	References
<i>Satureja montana</i>	Gentamicin	<i>E. coli</i> , <i>S. aureus</i>	Synergy/ Additive	(18)
<i>Origanum vulgare</i>	Norfloxacin	<i>S. aureus</i>	Synergy ↓MIC	(40)
<i>Thymus vulgaris</i>	Ciprofloxacin	Gram - bacteria	Synergy	(42)
<i>Ocimum basilicum</i>	Imipenem / Ciprofloxacin	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Synergy ↓MIC	(43)
<i>Cinnamomum verum</i>	Ampicillin / Chloramphenicol	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i>	Synergy ↓MIC	(44)
<i>Syzygium aromaticum</i>	Ampicillin/ Gentamicin	Oral bacteria	Synergy/ Additive	(45)
<i>Zataria multiflora</i>	Ciprofloxacin	Ciprofloxacin-resistant <i>P. aeruginosa</i>	Synergy ↓MIC	(34)
<i>Lavandula angustifolia</i>	Gentamicin	<i>S. aureus</i> , <i>S.aureus</i> MRSA	Synergy ↓MIC	(46)

values and faster elimination of pathogens (18,40,41). This opens promising perspectives for their use as adjuvants in antimicrobial therapy, particularly against multidrug-resistant bacteria, where reducing the effective antibiotic dose is of clinical importance.

For instance, the addition of *Satureja montana* essential oil to gentamicin has been shown to increase the susceptibility of *Escherichia coli* and

**Note:** MIC indicates a reduction in the minimum inhibitory concentration (MIC) of the antibiotic when combined with the essential oil. The data summarize synergistic interactions between essential oils and antibiotics, including *Satureja montana* essential oil combined with gentamicin against *E. coli* and *S. aureus* (18); *Origanum vulgare* essential oil combined with Norfloxacin against *S. aureus* (40); *Thymus vulgaris* essential oil combined with

Ciprofloxacin against *Shigella flexneri* (42); *Ocimum basilicum* essential oil combined with Imipenem or Ciprofloxacin against *S. aureus*, *E. coli*, and *P. aeruginosa* (43); *Cinnamomum verum* essential oil combined with Ampicillin or Chloramphenicol against *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (44); *Syzygium aromaticum* essential oil combined with Ampicillin or Gentamicin against oral bacteria (45); *Zataria multiflora* essential oil combined with Ciprofloxacin against Ciprofloxacin-resistant *P. aeruginosa* (34); and *Lavandula angustifolia* essential oil combined with Gentamicin against *S. aureus* (including MRSA) (46). These findings highlight the potential of combining essential oils with conventional antibiotics as an effective strategy against resistant pathogens.

### Antioxidant Activity

The antioxidant activity of essential oils has been extensively investigated using various in vitro methods, most commonly radical-scavenging assays such as DPPH and ABTS, as well as the ferric reducing antioxidant power (FRAP) assay. The results indicate that essential oils rich in phenolic constituents (such as eugenol, thymol, and carvacrol), as well as those containing high levels of oxygenated terpenes (e.g., thujone-containing oils), exhibit strong in vitro antioxidant activity (20,33,48). For example, oregano essential oil shows a low inhibitory concentration (IC<sub>50</sub>) in the DPPH assay, indicating high antioxidant activity due to its high content of carvacrol and thymol, whereas sage (*Salvia* spp.) essential oil, characterized by ketone components such as thujone, exhibits moderate antioxidant activity (7,49). It should

be noted that results obtained from different antioxidant assays are not always directly comparable, as each method is based on different mechanisms of action. Consequently, the same essential oil may demonstrate high activity in the DPPH assay but only moderate activity in the FRAP assay.

Importantly, the antioxidant effect of essential oils cannot be attributed to a single dominant component but rather results from complex interactions among multiple constituents. Essential oils are multicomponent systems in which both major and minor compounds contribute to the overall biological activity through synergistic or additive interactions (1,4,50). Similar observations have been reported for phenolic-rich essential oils such as thyme and oregano, where isolated major constituents (e.g., thymol or carvacrol) may display different activity compared to the complete essential oil, due to interactions with other terpenes and phenolic compounds (48). Consequently, the assessment of the antioxidant potential of essential oils requires consideration of the entire compositional profile rather than focusing solely on individual constituents. This highlights the limitations of reductionist approaches based exclusively on isolated compounds, as in many cases the complete essential oil exhibits greater biological activity than its individual components (1,4).

Table 4 presents results on the antioxidant activity of selected essential oils evaluated using different methods (DPPH, ABTS, FRAP). High antioxidant activity (low IC<sub>50</sub> values or strong radical-scavenging capacity) is observed for oregano, thyme, winter savory (*Satureja*), and clove essential oils, all of which

Table 4. Antioxidant activity of essential oils.

Essential Oil	Method	Activity	References
<i>Origanum vulgare</i>	DPPH	High	(20,47)
<i>Thymus vulgaris</i>	ABTS	High	(20)
<i>Rosmarinus officinalis</i>	FRAP	Moderate	(7,47,54)
<i>Salvia officinalis</i>	DPPH	Moderate	(55)
<i>Lavandula angustifolia</i>	DPPH	Moderate	(54)
<i>Zataria multiflora</i>	DPPH	High	(29)
<i>Ocimum basilicum</i>	DPPH	Moderate	(47)
<i>Satureja cuneifolia</i>	ABTS	High	(56)
<i>Cinnamomum verum</i>	DPPH	Moderate	(32)
<i>Syzygium aromaticum</i>	FRAP	High	(47,48)

are rich in phenolic terpenoid compounds (51,52,53). Moderate activity is characteristic of lavender and sage essential oils, which contain high proportions of linalool and ketones (weaker antioxidants). Rosemary essential oil shows variable results—some extracts and oils demonstrate strong FRAP capacity (attributed to rosmarinic acid), while others exhibit moderate activity depending on the chemotype (54). These variations further emphasize the influence of chemical composition on the antioxidant potential of essential oils.

**Note:** High activity indicates that the essential oil is able to neutralize a large proportion of free radicals at low concentrations (e.g.,  $IC_{50} < 0.1$  mg/mL in the DPPH assay). Moderate activity refers to higher  $IC_{50}$  values (0.1–0.5 mg/mL) or intermediate reducing capacity. The data are compared with literature references: for example, oregano essential oil exhibits strong DPPH radical scavenging capacity (20,47); thyme oil shows strong ABTS<sup>+</sup> radical neutralization (20); rosemary oil demonstrates moderate FRAP activity (except for extracts enriched in rosmarinic acid) (32); and clove oil displays high ferric reducing antioxidant power (FRAP) (47,48). These differences reflect both compositional variability and the specific mechanisms assessed by each antioxidant method.

### Antitumor Activity

In recent years, an increasing number of publications have reported the in vitro antitumor activity of essential oils and their constituents. Studies conducted on various tumor cell lines demonstrate that essential oils are capable of inducing apoptosis (programmed cell death), causing cell cycle arrest, and increasing the production of reactive oxygen species (ROS) in cancer cells (57,58,60). For example, oregano essential oil induces apoptosis in breast cancer cells (MCF-7) via a mitochondrial pathway, whereas thyme essential oil increases intracellular ROS levels and promotes oxidative stress in cervical carcinoma cells (HeLa) (57,58). Cinnamon essential oil (*C. verum*) exhibits cytotoxic effects against leukemic cells (HL-60) by inducing cell cycle arrest at the G<sub>0</sub>/G<sub>1</sub> phase (60). Lavender essential oil has been shown to exert a pronounced antiproliferative effect on lung carcinoma cells (A549), suppressing their growth and metabolic activity (59).

An interesting observation is that whole essential oils often display stronger antitumor effects than any of their major constituents tested individually, which is indicative of synergistic interactions among the components—similar to those described for antioxidant activity (60). For instance, clove essential oil, a complex mixture dominated by eugenol along with other terpenoid constituents, induces more pro-

Table 5. Antitumor activity of essential oils in vitro.

Essential Oil	Cell Line	Main Effect	References
<i>Origanum vulgare</i>	MCF-7 (Breast cancer)	Apoptosis	(62)
<i>Thymus vulgaris</i>	HeLa (Cervical carcinoma)	Reduced cell viability	(63)
<i>Cinnamomum zeylanicum</i>	HCT116 (Colorectal carcinoma)	Apoptosis + cell cycle arrest (G1 phase)	(64)
<i>Lavandula angustifolia</i>	A549 (Lung, adenocarcinoma)	Antiproliferation+ Apoptosis	(65)
<i>Melaleuca alternifolia</i>	Melanoma cells	Apoptosis+ reduced cell viability	(66)
<i>Boswellia sacra</i>	PC-3 (Prostate cancer)	Mitochondrial dysfunction	(67)
<i>Cymbopogon citratus</i>	HepG2 (Hepatocellular carcinoma)	ROS ↑ (oxidative stress)	(68)
<i>Syzygium aromaticum</i>	HT-29 (Colorectal carcinoma)	Apoptosis+ mitochondrial dysfunction (ROS ↑)	(61)
<i>Zingiber officinale</i>	SiHa (Cervical carcinoma)	Apoptosis (caspase-3 activation, mitochondrial pathway)	(69)
<i>Nigella sativa</i>	4T1 (Triple-negative breast cancer)	Anti-migration + MMP-9 inhibition (ROS ↑)	(70)

nounced apoptosis in colorectal cancer cells (HT-29) compared to pure eugenol alone (61).

An important aspect in the interpretation of antitumor data is that nearly all available evidence to date originates from *in vitro* experiments. Tumor cell lines are commonly used to assess cytotoxic effects of essential oils at defined concentrations; however, these concentrations are not always achievable *in vivo*, and cancer cells in *in vitro* models are generally more sensitive than tumors in living organisms. Therefore, although the results are promising—such as the complete inhibition of growth of triple-negative breast cancer cells by *Nigella sativa* essential oil (57)—they should be interpreted with caution, and a clear distinction must be made between experimental potential and clinical applicability. Table 5 summarizes data on the effects of selected essential oils on tumor cells, indicating the cell line used and the predominant observed effect (e.g., apoptosis, cell cycle arrest, oxidative stress).

**Note:** The reported effects were observed following exposure of tumor cells to essential oil concentrations typically ranging from 50 to 200 µg/mL, depending on cytotoxic thresholds (60). Apoptosis is confirmed through morphological changes (chromatin condensation, formation of apoptotic bodies) and specific markers (caspase activation, externalization of phosphatidylserine) (60). Cell cycle arrest commonly results in inhibition of cell division (60). ↑ROS denotes increased intracellular reactive oxygen species, which may cause oxidative damage and cell death (58). Mitochondrial dysfunction observed in PC-3 (prostate cancer) cells treated with *Boswellia* oil involves disruption of mitochondrial membrane potential and release of pro-apoptotic factors (67). Overall, significant cytotoxic effects against diverse cancer cell lines have been reported for essential oils from oregano, clove, thyme, cinnamon, lavender, tea tree, frankincense, lemongrass, ginger, and black cumin (33–35,42–48,61,64–72).

## DISCUSSION

The present review clearly demonstrates that essential oils are unique multitarget bioactive systems whose efficacy can not be reduced to the action of single components (4,6,60). Synergistic interactions between major and minor constituents explain both the broad spectrum of biological activity

and the relatively low tendency for the development of resistance in pathogens (4,6). Numerous studies using infectious disease models show that whole essential oils (e.g., thyme, oregano, clove) exhibit stronger antimicrobial activity than isolated individual components applied at equivalent concentrations (2,18,37,71). For example, *Thymus vulgaris* essential oil shows a more potent effect against *Staphylococcus aureus* compared with the same concentration of pure thymol (17,30). This can be explained by complementary mechanisms of action: phenolic monoterpenes disrupt cell membranes, terpene hydrocarbons facilitate their penetration, while oxygenated compounds (aldehydes, ketones) induce additional cellular stress (28,39). Similar synergistic effects have also been reported with respect to antifungal activity, where essential oils exert multitarget effects against fungal cells and spores (5,60,72,73). Of particular interest is the combination of different essential oils; several studies indicate that oil mixtures (e.g., tea tree + eucalyptus, thyme + rosemary) show stronger activity against MRSA and other pathogens than individual oils alone (6,74,75). This further supports the concept of *synergy within synergy*, referring not only to intracomponent synergy within a single oil, but also to synergistic interactions between different oils when applied in combination.

One of the central conclusions of this review is that GC–MS profiling is an essential prerequisite for the correct interpretation of the biological activity of essential oils. The lack of detailed chemical characterization is among the main reasons for contradictory data reported in the literature. For example, differences in the antimicrobial effects of products labeled as thyme oil are often due to different chemotypes—thymol-type versus linalool-type, the latter exhibiting weaker antibacterial potential (16,20,21). Similarly, if one study employs *Salvia officinalis* oil rich in thujone and camphor (ketone chemotype), while another uses a linalool-rich chemotype, their antioxidant activity results will differ significantly (7,26,29). This highlights the need for standardization of both analytical and biological methodologies in essential oil research. Although international standards (e.g., ISO and the European Pharmacopoeia) define purity and composition criteria for certain essential oils, unified requirements are lacking for most species. This review emphasizes the need to

implement standardized GC–MS protocols and reference chemotypes, which would improve inter-laboratory comparability and allow a more reliable correlation between chemical profiles and bioactivity.

Essential oils demonstrate considerable potential as natural antimicrobial agents. They are effective against a broad range of bacteria, including resistant strains, yeasts (*Candida* spp.), and even certain viruses (9,31,72). From a pharmacological perspective, however, their use as adjuvants to conventional antibiotics appears more realistic than their application as standalone antimicrobial agents. Several reasons support this view: first, achieving bactericidal effects in vivo often requires higher concentrations that may be difficult to reach systemically; second, some oils exhibit cytotoxic effects on human cells at elevated doses; third, compositional variability complicates dosage standardization. In this context, combining low doses of essential oils with antibiotics is particularly promising, as synergistic effects may restore or enhance antibiotic activity through complementary mechanisms such as biofilm disruption or inhibition of efflux pumps. This concept is supported by several recent in vitro and in vivo studies (6,9,18). Consequently, essential oils may serve as adjunctive therapies in infections—especially cutaneous and mucosal infections where topical application is feasible—as well as natural preservatives in foods to control pathogens without synthetic additives (72). While further safety and interaction studies are required, existing data indicate a generally favorable safety profile for many essential oils when used topically or by inhalation, with the exception of certain irritating phenols and ketones.

Many essential oils exhibit strong in vitro antioxidant activity, making them promising natural antioxidants for food, cosmetic, and dietary supplement applications (7,48,49). For example, the addition of clove or thyme essential oil significantly delays lipid oxidation in fat-rich foods by neutralizing free radicals. However, under in vivo conditions, the antioxidant effects of essential oils are more difficult to assess. Their small lipophilic molecules may act as pro-oxidants at high concentrations, and rapid in vivo metabolism limits their direct antioxidant activity in the human body. Rather, their relevance may lie in the modulation of endogenous antioxidant pathways, such as activation of the Nrf2 signaling

pathway—mechanisms that have been suggested for components like carvacrol and eugenol (5,53). From a practical standpoint, plant extracts and essential oils are already used as antioxidants in the food industry; for instance, extracts of rosemary and sage oils are approved as natural antioxidant additives. This review confirms that these oils exhibit measurable in vitro antioxidant capacity while emphasizing its complex nature—it cannot be attributed to a single molecule, but rather to the entire phytochemical complex (23,28).

Reported anticancer effects of essential oils are particularly promising, especially considering that many of their mechanisms—apoptosis induction, inflammation suppression, and antioxidant activity—target key processes involved in carcinogenesis (57,60). For example, certain terpenes (linalool, geraniol) enhance tumor cell sensitivity to chemotherapy, while others (eugenol, citral) exert direct cytotoxic effects on cancer cells while sparing normal cells (33,61). Nevertheless, it must be emphasized that available data are predominantly preclinical, consisting mainly of in vitro experiments and animal studies. There is a lack of sufficient clinical trials in humans to demonstrate the efficacy and safety of essential oils as anticancer agents. Due to their volatile and unstable nature, achieving therapeutic concentrations in tumor tissue remains challenging. Potential solutions include the development of nanoformulations, emulsions, or targeted delivery systems to enhance bioavailability of anticancer constituents (10,50). Additionally, combining essential oils or their active compounds with standard chemotherapeutics may yield adjuvant effects; for example, the combination of D-limonene (from citrus oil) with doxorubicin results in enhanced apoptosis in breast cancer cells compared to monotherapy. A major limitation remains potential toxicity—some oils containing thujone or safrole are associated with adverse effects at high doses, including neurotoxicity and genotoxicity—necessitating careful safety evaluation for long-term use.

In conclusion, accumulated evidence clearly positions essential oils as promising multifunctional agents with antimicrobial, antioxidant, and anticancer potential. Their multi-target mechanisms represent both a strength (synergistic effects and reduced resistance development) and a challenge (analytical

complexity and compositional variability). Future applications will depend on the ability of the scientific community to standardize products, demonstrate *in vivo* efficacy and clinical relevance, and ensure safety. In the context of rising antibiotic resistance, the demand for new antioxidants, and the shift toward nature-based therapies, essential oils have the potential to become valuable complementary tools in modern medicine and industry (9,10,51,52). A substantial body of literature already supports these perspectives and provides a solid foundation for future research (53–63,72–74).

## CONCLUSION

Essential oils are multitarget bioactive systems with demonstrated antimicrobial, antioxidant, and anticancer potential, driven by their complex and variable chemical composition. GC–MS profiling is crucial for the accurate interpretation of their biological activity, as chemotypic and geographic variability significantly influence efficacy. Current evidence suggests that essential oils may serve as promising natural adjuvants to antibiotic therapy, as well as sources of natural antioxidants and potential anticancer agents, although most data remain *in vitro*. Further standardized *in vivo* and clinical studies are required to fully assess their efficacy, safety, and applicability in medicine and industry.

## Acknowledgments

The research study was financed with funds from the state budget, provided through the Ministry of Education and Science (MES) to the Science Fund at the Medical University - Varna for financing the scientific activity inherent in state higher education institutions under project № 24027.

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