

Volatile Antimicrobial Agents and *In Vitro* Methods for Evaluating Their Activity in the Vapour Phase: A Review

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Key words

antibacterial, broth dilution, diffusion, essential oils, plant volatiles, volatilization

received November 8, 2019

revised March 19, 2020

accepted April 14, 2020

Bibliography

DOI <https://doi.org/10.1055/a-1158-4529>

published online May 25, 2020 | *Planta Med* 2020; 86: 822–857 © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

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ABSTRACT

This review summarizes data on the *in vitro* antimicrobial effectiveness of volatile agents of plant origin and *in vitro* meth-

ods for evaluating their activity in the vapour phase. As a result of literature analysis, the antimicrobial efficacy of vapours from 122 different plant species and 19 pure compounds examined in 61 studies using different *in vitro* tests against a broad spectrum of microorganisms was identified and summarized. In addition, 11 different techniques found in the literature are described in detail. An original classification of methods based on the solid and liquid matrix volatilization principle is proposed because carrier medium/matrix selection is crucial for the volatilization of any agents tested. This review should be useful for medicinal, pharmaceutical, food, and agricultural experts working in areas related to the management of infectious diseases (especially respiratory and skin infections), food preservation (active packaging), and protection of agriculture products (controlled atmosphere). It may also stimulate the interest of pharmaceutical, cosmetic, food, and agriculture industries in the research and development of new antimicrobial agents of natural origin. Since several original apparatuses previously developed for antimicrobial susceptibility testing in the vapour phase are described in this review, labware manufacturers may also be interested in this topic. The review also provides specific guidelines and recommendations for researchers studying the antimicrobial activity of volatile agents. The article will therefore appeal to communities of industrial stakeholders, pharmacists, physicians, food experts, agriculturists, and researchers in related areas such as pharmacology, medicinal chemistry, microbiology, natural product chemistry, food preservation and plant protection.

Introduction

Volatile agents (VAs) are abundant chemicals that are emitted by organisms in all terrestrial and marine ecosystems as an important factor, allowing communication and interaction among plants, microorganisms, animals, and the environment [1,2]. The primary functions of VAs are defence against herbivores and pathogens and the attraction of pollinators and seed dispersers, signalling involved in plant communication, and they also can act as wound sealers in some plants [3]. This relatively large group of natural products consisting of lipophilic compounds of low molecular weight and high vapour pressure at ambient temperatures is divided into several chemical classes, including terpenoids, phenylpropanoids, fatty acid derivatives, and amino acid-derived

products in addition to a few specific compounds not represented in these major classes such as alkanes, alkenes, alcohols, esters, aldehydes, and ketones of various biogenetic origin [4]. The physical properties of these VAs enable them to freely cross cellular membranes and be released into the surrounding environment [5].

In recent years, natural substances including VAs have been intensively studied for the purpose of reducing the utilization of synthetic antimicrobial agents in pharmacy, foods, and agriculture. They are considered relatively safe, and are easily decomposed, environmentally friendly, and non-phytotoxic [6]. Moreover, VAs have the benefit of being bioactive in their vapour phase. Thanks to this, volatiles have great potential for the development of novel agricultural, food, and pharmaceutical plant-derived products and technologies [7].

Among the most important representatives of VAs are essential oils (EOs) produced via plant metabolism. EOs are aromatic liquids of complex composition including aliphatic hydrocarbons, terpenoids, and phenylpropanoids [8]. Plant species producing EOs belong to various genera divided into around 60 families such as the Alliaceae, Apiaceae, Asteraceae, Chenopodiaceae, Cyperaceae, Lamiaceae, Lauraceae, Myrtaceae, Poaceae, Piperaceae, Rutaceae, Verbenaceae, and Zingiberaceae [9, 10]. Commonly, they are obtained via the distillation and cold pressing of whole plants or their individual parts (bark, buds, flowers, fruits, leaves, roots, seeds, stems, and wood) [11]. However, enfleurage, organic solvent, and supercritical fluid extractions are another possible way of isolating products containing volatile compounds such as oleoresins. The extraction process plays a vital role in the yield and quality of EOs and related products [12]. Since ancient times, EOs have been widely used in medicine, perfumery, cosmetics, and the food industry. As they often possess a broad spectrum of biological effects [13], EOs have potential in various applications for human, animal, and plant health, as well as food quality. Besides antimicrobial activity [14–16], EOs and their constituents have been observed to exhibit anaesthetic [17], antiparasitic [18], anti-inflammatory [19], antioxidant [20], antiulcer [21], antiviral [22], cytotoxic [23], immunomodulatory [24], insecticidal [25], and molluscicidal [26] properties. Despite their historical record as medicinal agents, European and American health authorities such as the European Pharmacopoeia and the United States Pharmacopoeia National Formulary list only a small number of EO-bearing plants and their preparations as medicinal [3].

Their main advantages are that they do not need to be applied systemically to the body or directly to agricultural or food products and that they naturally tend to be regularly distributed in the air conditions of the target area. In addition, EOs are a typical example of complex mixtures producing an antimicrobial synergistic effect, which is currently considered an effective tool in overcoming microbial resistance [27]. It should nevertheless be noted that respiratory, allergic, and immune effects have been associated with the inhalation of EOs, especially in infants and children. Therefore, a detailed safety evaluation of novel plant-derived VAs is necessary before their introduction in practical use [28].

Antimicrobial Activity of Volatiles

Currently, various over-the-counter pharmaceuticals, cosmetics, and herbal medicines containing EOs derived from plants (e.g., *Melaleuca alternifolia*) and their volatile compounds (e.g., eucalyptol and thymol) with proven clinical efficacy are used for the prevention and treatment of oral, respiratory, and skin infections in humans [29]. A few food preservatives consisting of EOs from *Citrus* spp., *Rosmarinus officinalis*, and *Salvia officinalis* are also already commercially available for application in various food products [30]. In addition, fungicides containing EOs (e.g., *Citrus* spp., *Foeniculum vulgare*, *Mentha* spp., and *Thymus vulgaris*) and their volatile constituents (e.g., carvacrol, menthol, thymol) are currently used under greenhouse and field conditions in smallholder gardening and organic agriculture to grow healthy crops and to control postharvest decay in different horticultural commodities

[31–33]. However, industrial antimicrobial applications based on the most typical physicochemical feature of these agents, which is volatility, have not been fully developed yet. VAs could be applicable for the development of new pharmaceutical preparations (e.g., inhalation therapy), disinfection and sterilization agents in healthcare facilities, the protection of stored agricultural products (e.g., controlled atmosphere storage), and for the preservation and shelf-life extension of food products (e.g., active packaging) [34]. Only a few fumigants recommended for organic greenhouse application, e.g., Eco-Oil (Organic Crop Protectants), Prev-AM (Oro Agri International), Requiem (Bayer AG), and inhaler nasal sticks for ease of breathing (e.g., Vicks), based on the vapours of plant volatiles have been commercialized. These preparations are composed of ingredients such as camphor, limonene, menthol, and the EOs of *Abies sibirica*, *Citrus* sp., and *Melaleuca alternifolia* [35, 36].

As far as research on the *in vitro* antimicrobial activity of VAs in the vapour phase is concerned, their growth-inhibitory effect has been studied against a broad spectrum of pathogenic microorganisms associated with human infections (e.g., *Candida albicans*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*), foodborne diseases (e.g., *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis*), and plant diseases (e.g., *Alternaria alternata*, *Aspergillus* sp., *Fusarium oxysporum*, *Penicillium* sp.) [37]. The results of the antimicrobial effects of EOs and volatile compounds previously tested by various authors in a gaseous phase are summarized in ► **Tables 1 and 2**, respectively. According to previously published data, a high level of efficiency was observed for EOs isolated from plant species such as *Armoracia rusticana*, *Brassica nigra*, *Cinnamomum zeylanicum*, *Cymbopogon citratus*, *Lavandula* sp., *Lippia berlandieri*, *Origanum vulgare*, *Pulicaria mauritanica*, and *Syzygium aromaticum*, as well for pure compounds such as carvacrol, 8-hydroxyquinoline, linalool, linalyl acetate, α -pinene, and thymoquinone (see ► **Tables 1 and 2**). A disc volatilization method based on the evaporation of VAs from a solid matrix (e.g., paper disc) was the most commonly used assay method in the investigation of the antimicrobial potential of their vapours. However, a number of samples previously assayed by this method, which is suitable only for qualitative evaluation, were tested at relatively high concentrations (e.g., 800–1600 mg/L), which can lead to a misinterpretation of the importance for practical use of the results obtained. Besides, inaccurate VA specification, especially incomplete botanical data about the EOs' sources (such as plant species identification, the part of the plant processed), is another weakness affecting the comparability of some results described in the literature.

Methods for Evaluation of Growth Inhibitory Effects of the Vapour Phase

Before the introduction to practical use of new preparations based on VAs, a detailed evaluation of their efficiency and safety is necessary, whereas *in vitro* screening is usually the first step in this process. Among *in vitro* techniques, vapour phase tests demonstrate the antimicrobial activity of VAs in a way which respects their specific physicochemical properties such as high volatility,

► **Table 1** Antimicrobial activity of EO vapours tested *in vitro* against various microorganisms.

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Abies siberica</i>	Pinaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophaea apis</i>	MIC 500 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 500 µL/L	[55]
<i>Ageratum houstonianum</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 61.5% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 37.0% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 66.6% 0.33 µL/mL	[81]
<i>Allium sativum</i>	Amaryllidaceae	bulb	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 10.0 µL/mL	[63]
				<i>Penicillium corylophilum</i>	MIC 0.0390 µL/mL	[84]
			disc volatilization method	<i>Bacillus cereus</i>	IZ 33.0 mm for 10 µL of EO	[82]
				<i>Escherichia coli</i>	MIC 530 µL/L	[54]
				<i>Listeria monocytogenes</i>	MIC 8.3 µL/L	[54]
				<i>Pseudomonas aeruginosa</i>	MIC 530 µL/L	[54]
				<i>Salmonella enteritidis</i>	MIC 260 µL/L	[54]
				<i>Staphylococcus aureus</i>	MIC 8.3–530.0 µL/L	[54, 83]
			multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 31.25 µL/L	[55]
				<i>Ascophaea apis</i>	MIC 63–125 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 31.25 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 31.25 µL/L	[55]
		<i>Pseudomonas aeruginosa</i>		MIC 250 µL/L	[55]	
		<i>Staphylococcus aureus</i>		MIC 250 µL/L	[55]	
		root	airtight apparatus disc volatilization method	<i>Penicillium corylophilum</i>	MIC 0.0390 µL/L	[84]
		not specified	disc volatilization method	<i>Bacillus cereus</i>	IZ 90.0 mm for 5 µL of EO	[85]
				<i>Clostridium perfringens</i>	IZ 17.17–17.85 mm for 5 µL of EO	[85]
				<i>Pseudomonas fluorescens</i>	IZ 23.42–27.25 mm for 5 µL of EO	[85]
				<i>Salmonella typhimurium</i>	IZ 10.50–27.75 mm for 5 µL of EO	[85]
<i>Staphylococcus aureus</i>	IZ 22.25–90.00 mm for 5 µL of EO			[85]		
<i>Alpinia cumingii</i>	Zingiberaceae	leaf	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 256 µg/mL	[86]
<i>Alpinia oxymitra</i>	Zingiberaceae	leaf	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 128 µg/mL	[87]
		pericarp		<i>Haemophilus influenzae</i>	MIC 8 µg/mL	[87]
		rhizome		<i>Haemophilus influenzae</i>	MIC 16 µg/mL	[87]
		seed		<i>Haemophilus influenzae</i>	MIC 64 µg/mL	[87]
				<i>Staphylococcus aureus</i>	MIC 256 µg/mL	[87]
<i>Amyris balsamifera</i>	Rutaceae	not specified	multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Anisomeles indica</i>	Lamiaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 19.2% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 17.3% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 44.9% 0.33 µL/mL	[81]

Continued

► **Table 1** *Continued*

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Armoracia rusticana</i>	Brassicaceae	root	disc volatilization method	<i>Bacillus amyloliquefaciens</i>	MIC 0.039 mg/mL; IZ 87.00 mm	[88]
				<i>Bacillus pumilus</i>	MIC 0.039 mg/mL; IZ 87.00 mm	[88]
				<i>Enterobacter amnigenus</i>	MIC 0.052 mg/mL; IZ 24.12 mm	[88]
				<i>Escherichia. coli</i>	MIC 8.3 µL/L	[54]
				<i>Listeria monocytogenes</i>	MIC 8.3 µL/L	[54]
				<i>Pseudomonas aeruginosa</i>	MIC 8.3 µL/L	[54]
				<i>Salmonella enteritidis</i>	MIC 8.3 µL/L	[54]
				<i>Staphylococcus aureus</i>	MIC 8.3–17.0 µL/L	[54, 83]
				<i>Staphylococcus xylosus</i>	MIC 0.039 mg/mL; IZ 31.92 mm	[88]
			<i>Streptococcus</i> sp.	MIC 1.563 mg/mL; IZ 8.70 mm	[88]	
			multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 31.25 µL/L	[58]
				<i>Ascophæra apis</i>	MIC 16 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 31.25 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 31.25 µL/L	[55]
				<i>Pseudomonas aeruginosa</i>	MIC 31.25 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 31.25 µL/L	[55]
<i>Staphylococcus aureus</i>	MIC 31.25 µL/L	[55]				
<i>Artemisia annua</i>	Asteraceae	not specified	disc volatilization method	<i>Candida albicans</i>	IZ 8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida dubliniensis</i>	IZ 8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida glabrata</i>	IZ 8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida krusei</i>	IZ 8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida norvegensis</i>	IZ 8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida parapsilosis</i>	IZ 4.4–8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Candida tropicalis</i>	IZ 6.8–8.5 cm for 0.32–0.64 µL/cm ³ of EO	[89]
				<i>Malassezia furfur</i>	O 100% for 0.133–0.530 µL/cm ³ of air	[90]
				<i>Malassezia globosa</i>	O 100% for 0.066–0.133 µL/cm ³ of air	[90]
				<i>Malassezia pachydermatis</i>	O 100% for 0.066 µL/cm ³ of air	[90]
				<i>Malassezia sloffiae</i>	O 100% for 0.066–0.530 µL/cm ³ of air	[90]
				<i>Malassezia sympodialis</i>	O 100% for 0.133–0.530 µL/cm ³ of air	[90]
<i>Artemisia nilagirica</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 100% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 100% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 100% 0.33 µL/mL	[81]

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Betula lenta</i>	Betulaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophaera apis</i>	MIC 500 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 500 µL/L	[55]
<i>Blumea lacera</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 18.1% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 42.6% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 32.2% 0.33 µL/mL	[81]
<i>Blumea laciniata</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 17.8% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 23.5% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 20.0% 0.33 µL/mL	[81]
<i>Blumea membranacea</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 60.0% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 56.0% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 46.2% 0.33 µL/mL	[81]
<i>Boesenbergia rotunda</i>	Zingiberaceae	rhizome	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 256 µg/mL	[87]
<i>Boswellia carteri</i>	Burseraceae	frankincense	disc volatilization method	<i>Aspergillus europaeus</i>	O 28.8–100.0% 4–12 mg/cm ³	[91]
				<i>Aspergillus flavus</i>	O 30.8–80.8% 4–12 mg/cm ³	[91]
				<i>Aspergillus niger</i>	O 16.4–80.8% 4–12 mg/cm ³	[91]
				<i>Cladosporium cladosporioides</i>	O 100% 4–12 mg/cm ³	[91]
				<i>Cladosporium uredinicola</i>	O 100% 4–12 mg/cm ³	[91]
				<i>Penicillium atosanguineum</i>	O 53.4–86.3% 4–12 mg/cm ³	[91]
				<i>Penicillium bilaiae</i>	O 72.6–95.9% 4–12 mg/cm ³	[91]
				<i>Penicillium lanosum</i>	O 79.5–98.6% 4–12 mg/cm ³	[91]
<i>Brassica nigra</i>	Brassicaceae	not specified	disc volatilization method	<i>Acinetobacter baumannii</i>	MIC 0.012 µg/mL	[92]
				<i>Aspergillus fumigatus</i>	MIC 0.025 µg/mL	[92]
				<i>Aspergillus niger</i>	MIC 0.020 µg/mL; 3.08 µL/L _{air}	[91, 93]
				<i>Aspergillus nomius</i>	MIC 0.060 µg/mL	[92]
				<i>Bacillus subtilis</i>	MIC 0.250 µg/mL	[92]
				<i>Candida albicans</i>	MIC 0.012 µg/mL	[92]
				<i>Cryptococcus neoformans</i>	MIC 0.060 µg/mL	[92]
				<i>Escherichia coli</i>	MIC 0.050 µg/mL	[92]
				<i>Eupenicillium hirayamae</i>	MIC 0.060 µg/mL	[92]
				<i>Mycobacterium smegmatis</i>	MIC 0.012 µg/mL	[92]
				<i>Penicillium cinnamopurpureum</i>	MIC 0.025 µg/mL	[92]
				<i>Penicillium expansum</i>	MIC 0.012 µg/mL	[92]
				<i>Penicillium viridicatum</i>	MIC 0.060 µg/mL	[92]
				<i>Pseudomonas aeruginosa</i>	MIC 0.012 µg/mL	[92]
				<i>Salmonella typhimurium</i>	MIC 0.025 µg/mL; IZ 57.5–85.0 mm for 10 µL of EO	[91, 94]
				<i>Staphylococcus aureus</i>	MIC 0.012 µg/mL	[92]
				<i>Streptococcus pyogenes</i>	MIC 0.012 µg/mL	[92]
<i>Trichophyton rubrum</i>	MIC 0.050 µg/mL	[92]				

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Cannabis sativa</i>	Cannabinaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 40.0% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 48.5% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 38.4% 0.33 µL/mL	[81]
<i>Caryopteris × clandonensis</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]
<i>Cinnamomum</i> sp.	Lauraceae	bark	disc volatilization method	<i>Salmonella typhimurium</i>	IZ 7.50–83.75 mm or 10 µL of EO	[94]
<i>Cinnamomum cambodianum</i>	Lauraceae	bark	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 256 µg/mL	[87]
		leaf		<i>Haemophilus influenzae</i>	MIC 128 µg/mL	[87]
<i>Cinnamomum cassia</i>	Lauraceae	bark	disc volatilization method	<i>Escherichia coli</i>	IZ 67.5 mm for 30 µL of EO	[95]
				<i>Listeria monocytogenes</i>	IZ 73.0 mm for 30 µL of EO	[95]
				<i>Pseudomonas aeruginosa</i>	IZ 27.8 mm for 30 µL of EO	[95]
				<i>Salmonella typhimurium</i>	IZ 32.7 mm for 30 µL of EO	[95]
				<i>Staphylococcus aureus</i>	IZ 73.0 mm for 30 µL of EO	[95]
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 31.25 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 125 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 31.25 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 62.5 µL/L	[55]
				<i>Pseudomonas aeruginosa</i>	MIC 500 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 500 µL/L	[55]
<i>Cinnamomum verum</i>	Lauraceae	bark	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 0.0391 µL/mL	[63]
				<i>Penicillium corylophilum</i>	MID 0.1563 µL/L	[84]
		airtight box disc volatilization method	<i>Escherichia coli</i>	MID 12.5 mg/L	[53]	
			<i>Haemophilus influenzae</i>	MID 3.13 mg/L	[53]	
			<i>Staphylococcus aureus</i>	MID 6.25 mg/L	[53]	
			<i>Streptococcus pneumoniae</i>	MID 1.56–3.13 mg/L	[53]	
			<i>Streptococcus pyogenes</i>	MID 6.25 mg/L	[53]	
		disc volatilization method	<i>Aspergillus flavus</i>	MIC 13.1 µL/L	[57]	
			<i>Aspergillus niger</i>	MIC 5.625 µg/mL	[56]	
			<i>Bacillus megaterium</i>	MIC 11.25 µg/mL	[56]	
			<i>Candida albicans</i>	MIC 13.1 µL/L	[57]	
			<i>Colletotrichum gloeosporioides</i>	O 49.2–100.0% 1–8 µL EO per PD	[52]	
			<i>Escherichia coli</i>	MIC 17.5–128.0 µL/L; IZ 45.0 mm for 30 µL of EO	[57, 95, 96]	
			<i>Lasiodiplodia theobromae</i>	O 57.1–100.0% 1–8 µL EO per PD	[52]	
			<i>Listeria monocytogenes</i>	IZ 52.8 mm for 30 µL of EO	[95]	
			<i>Penicillium funiculosum</i>	MIC 5.625 µg/mL	[56]	
			<i>Penicillium islandicum</i>	MIC 8.73 µL/L	[57]	
			<i>Pseudomonas fluorescens</i>	MIC 22.50 µg/mL	[56]	
			<i>Salmonella enteritidis</i>	MIC 512 µL/L	[96]	
<i>Salmonella choleraesuis</i>	MIC 131.0 µL/L	[57]				
<i>Salmonella typhimurium</i>	IZ 12.3 mm for 30 µL of EO	[95]				

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
			multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	IZ 35.4 mm for 30 µL of EO	[95]
				<i>Streptomyces rutgersensis</i>	MIC 22.50 µg/mL	[56]
				<i>Trichoderma viride</i>	MIC 11.25 µg/mL	[56]
				<i>Yersinia enterocolitica</i>	MIC 17.5 µL/L	[57]
				<i>Alternaria alternata</i>	MIC 32 µL/L	[97]
				<i>Aspergillus niger</i>	MIC 128 µL/L	[97]
				<i>Botrytis cinerea</i>	MIC 32 µL/L	[97]
				<i>Cladosporium cucumerinum</i>	MIC 64 µL/L	[97]
				<i>Claviceps purpurea</i>	MIC 16 µL/L	[97]
				<i>Dendryphion penicillatum</i>	MIC 64 µL/L	[97]
				<i>Helminthosporium solani</i>	MIC 32 µL/L	[97]
				<i>Monilia fructigena</i>	MIC 64 µL/L	[97]
				<i>Penicillium digitatum</i>	MIC 256 µL/L	[97]
				<i>Penicillium expansum</i>	MIC 32 µL/L	[97]
				<i>Phoma foveata</i>	MIC 32 µL/L	[97]
		<i>Pseudomonas aeruginosa</i>	MIC 31.25–125.00 µL/L	[87]		
		<i>Staphylococcus aureus</i>	MIC 31.25 µL/L	[98]		
		leaf	agar plug-based vapor phase assay	<i>Histophilus somni</i>	O complete inhibition	[50]
				<i>Mannheimia haemolytica</i>	O 5 log ₁₀ CFU for 100 µL of EO	[50]
				<i>Pasteurella multocida</i>	O 3 log ₁₀ CFU for 100 µL of EO	[50]
		not specified	disc volatilization method	<i>Bacillus cereus</i>	IZ 12.3 mm for 10 µL of EO	[82]
<i>Acinetobacter baumannii</i>	IZ 19–30 mm for 100 µL of EO			[99]		
<i>Bacillus cereus</i>	MIC 17.5 µL/L; IZ 26 mm for 10 µL of EO			[57, 60]		
<i>Bacillus subtilis</i>	IZ 43 mm for 100 µL of EO			[99]		
<i>Enterococcus faecalis</i>	MIC 52.4 µL/L; IZ 15 mm for 10 µL of EO			[57, 60]		
<i>Escherichia coli</i>	MIC 10 mg/L; IZ 13–22 mm for 10–100 µL of EO			[60, 99, 100]		
<i>Listeria monocytogenes</i>	MIC 34.9 µL/L; IZ 13 mm for 10 µL of EO			[57, 60]		
<i>Salmonella choleraesuis</i>	IZ 26 mm for 10 µL of EO			[60]		
<i>Staphylococcus aureus</i>	MIC 20 mg/L; 34.9 µL/L; IZ 24 mm for 10 µL of EO			[57, 60, 100]		
<i>Yersinia enterocolitica</i>	IZ 32 mm for 10 µL of EO	[60]				
<i>Cistus ladaniferus</i>	Cistaceae	leaf and branch	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 2.50 µL/mL	[63]
<i>Citrus × aurantium</i>	Rutaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
<i>Citrus bergamia</i>	Rutaceae	not specified	disc volatilization method	<i>Bacillus cereus</i>	IZ 28 mm	[101]
				<i>Listeria monocytogenes</i>	IZ 54 mm	[101]
				<i>Staphylococcus aureus</i>	IZ 26 mm	[101]
<i>Citrus limon</i>	Rutaceae	leaf	disc volatilization method	<i>Listeria monocytogenes</i>	MIC 0.086 µL/cm ³	[102]

Continued

► **Table 1** *Continued*

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference			
<i>Citrus lucida</i>	Rutaceae	fruit peel	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 64 µg/mL	[87]			
				<i>Staphylococcus aureus</i>	MIC 256 µg/mL	[87]			
<i>Citrus sinensis</i>	Rutaceae	fruit peel	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 17.8–78.8 mm for 10 µL of EO	[103]			
<i>Citrus</i> sp.	Rutaceae	not specified	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 200 mg/L air	[53]			
				<i>Staphylococcus aureus</i>	MID 800 mg/L air	[53]			
				<i>Streptococcus pneumoniae</i>	MID 400 mg/L air	[53]			
				<i>Streptococcus pyogenes</i>	MID 200 mg/L air	[53]			
<i>Coriandrum sativum</i>	Apiaceae	fruit	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 12.5 mg/L air	[53]			
				<i>Streptococcus pneumoniae</i>	MID 25 mg/L air	[53]			
				<i>Streptococcus pyogenes</i>	MID 25 mg/L air	[53]			
		not specified	airtight box disc volatilization method	<i>Escherichia coli</i>	MID 50 mg/L	[53]			
				multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]		
			<i>Ascophæra apis</i>		MIC 250–500 µL/L	[80]			
	<i>Aspergillus niger</i>		MIC 500 µL/L		[55]				
	Apiaceae	seed	disc volatilization method	<i>Penicillium digitatum</i>	MIC 500 µL/L	[55]			
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]			
				<i>Cuminum cyminum</i>	Apiaceae	seed	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 26.6 mm for 30 µL of EO
<i>Curcuma aromatica</i>				Zingiberaceae				rhizome	disc volatilization method
<i>Aspergillus niger</i>	O 24.3% 0.33 µL/mL	[81]							
<i>Aspergillus ochraceus</i>	O 42.8% 0.33 µL/mL	[81]							
<i>Cupressus sempervirens</i>	Cupressaceae	leaf and branch	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 5.00 µL/mL	[63]			
<i>Cymbopogon citratus</i>	Poaceae	aerial parts	airtight box disc volatilization method	<i>Escherichia coli</i>	MID 100 mg/L air	[53]			
				<i>Haemophilus influenzae</i>	MID 1.56 mg/L air	[53]			
				<i>Staphylococcus aureus</i>	MID 12.5 mg/L air	[53]			
				<i>Streptococcus pneumoniae</i>	MID 6.25 mg/L air	[53]			
				<i>Streptococcus pyogenes</i>	MID 6.25 mg/L air	[53]			
				leaf	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 5.00 µL/mL	[63]	
		disc volatilization method	<i>Bacillus cereus</i>			IZ 20.0 mm for 10 µL of EO	[104]		
			<i>Escherichia coli</i>	IZ 10.0–56.0 mm for 10–40 µL of EO	[104, 105]				
			<i>Listeria monocytogenes</i>	IZ 22.0 mm for 10 µL of EO	[104]				
			<i>Staphylococcus aureus</i>	IZ 20.0 mm for 10 µL of EO	[104]				
		not specified	disc volatilization method	<i>Candida albicans</i>	IZ 80–90 mm for 20–60 µL of EO	[106]			
				multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 256–500 µL/L	[55, 97]		
			<i>Aspergillus niger</i>		MIC 250–512 µL/L	[55, 97]			
			<i>Botrytis cinerea</i>		MIC 128 µL/L	[97]			
<i>Cladosporium cucumerinum</i>	MIC 256 µL/L		[97]						
<i>Claviceps purpurea</i>	MIC 128 µL/L		[97]						

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
				<i>Dendryphion penicillatum</i>	MIC 512 µL/L	[97]
				<i>Helminthosporium solani</i>	MIC 64 µL/L	[97]
				<i>Monilia fructigena</i>	MIC 256 µL/L	[97]
				<i>Penicillium digitatum</i>	MIC 500 µL/L	[55]
				<i>Phoma foveata</i>	MIC 64 µL/L	[97]
<i>Cymbopogon flexuosus</i>	Poaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125 µL/L	[55]
				<i>Ascophaea apis</i>	MIC 63 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 125 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 250 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Cymbopogon martini</i>	Poaceae	aerial parts	disc volatilization method	<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
		not specified	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 7.0 mm for 15 µL of EO	[107]
				<i>Staphylococcus epidermidis</i>	IZ 6.0 mm for 15 µL of EO	[107]
<i>Cymbopogon martini</i> var. <i>sofia</i>	Poaceae	leaf	agar plug-based vapor phase assay	<i>Histophilus somni</i>	O 5 log ₁₀ CFU for 100 µL of EO	[50]
				<i>Mannheimia haemolytica</i>	O 6 log ₁₀ CFU for 100 µL of EO	[50]
				<i>Pasteurella multocida</i>	O 7 log ₁₀ CFU for 100 µL of EO	[50]
<i>Cymbopogon nardus</i>	Poaceae	leaf	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]
				<i>Penicillium corylophilum</i>	MID 0.3125 µL/L	[84]
		not specified	disc volatilization method	<i>Colletotrichum gloeosporioides</i>	O 42.2–75.6% 1–5 µL EO per PD	[108]
				<i>Lasiodiplodia theobromae</i>	O 27.6–75.0% 1–5 µL EO per PD	[108]
				<i>Monilinia fructicola</i>	O 12.6–65.0% 1–5 µL EO per PD	[108]
				<i>Penicillium expansum</i>	O 32.3–72.3% 1–5 µL EO per PD	[108]
				<i>Rhizopus stolonifer</i>	O 28.7–78.0% 1–5 µL EO per PD	[108]
			multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	MIC 375 µL/L	[98]
<i>Daucus carota</i>	Apiaceae	not specified	multi-screening disc volatilization method	<i>Ascophaea apis</i>	MIC 500 µL/L	[80]
<i>Erigeron canadensis</i>	Asteraceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 55.5% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 35.8% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 49.3% 0.33 µL/mL	[81]
<i>Eucalyptus globulus</i>	Myrtaceae	not specified	disc volatilization method	<i>Candida albicans</i>	IZ 10–70 mm for 20–60 µL of EO	[106]
<i>Eucalyptus citriodora</i>	Myrtaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 32.2% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 23.2% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 30.0% 0.33 µL/mL	[81]

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Eucalyptus radiata</i>	Myrtaceae	leaf	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 25 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 200 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 50–100 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 50 mg/L air	[53]
<i>Eucalyptus</i> sp.	Myrtaceae	not specified	disc volatilization method	<i>Aureobasidium pullulans</i>	MIC 4.5 mg/mL	[109]
				<i>Candida diversa</i>	MIC 2.25 mg/mL	[109]
				<i>Hansenula polymorpha</i>	MIC 2.25 mg/mL	[109]
				<i>Pichia anomala</i>	MIC 1.13 mg/mL	[109]
				<i>Pichia fermentans</i>	MIC 2.25 mg/mL	[109]
				<i>Pichia kluyveri</i>	MIC 0.56 mg/mL	[109]
				<i>Saccharomyces cerevisiae</i>	MIC 4.5 mg/mL	[109]
				<i>Zygosaccharomyces bailii</i>	MIC 2.25 mg/mL	[109]
<i>Foeniculum vulgare</i> var. <i>dulce</i>	Apiaceae	not specified	disc volatilization method	<i>Candida albicans</i>	MIC 0.25–1.00 µg/mL	[110]
				<i>Candida glabrata</i>	MIC 0.25–1.00 µg/mL	[110]
				<i>Candida tropicalis</i>	MIC 0.25–1.00 µg/mL	[110]
<i>Hyptis suaveolens</i>	Lamiaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 58.5 % 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 57.2 % 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 48.1 % 0.33 µL/mL	[81]
<i>Hyssopus officinalis</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]
<i>Jasminum officinale</i>	Oleaceae	not specified	disc volatilization method	<i>Penicillium citrinum</i>	IZ 5.17–45.67 mm for 50 µL of EO	[111]
				<i>Penicillium crustosum</i>	IZ 16.17–42.50 mm for 50 µL of EO	[111]
				<i>Penicillium expansum</i>	IZ 16.00–35.83 mm for 50 µL of EO	[111]
<i>Juniperus communis</i>	Cupressaceae	not specified	multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Juniperus virginiana</i>	Cupressaceae	not specified	multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Lantana indica</i>	Verbenaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 27.0 % 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 18.9 % 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 50.8 % 0.33 µL/mL	[81]
<i>Laurus nobilis</i>	Lauraceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophaera apis</i>	MIC 500 µL/L	[80]
<i>Lavandula angustifolia</i>	Lamiaceae	flowery top	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 25 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MIC 100 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MIC 50 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MIC 50 mg/L air	[53]
		not specified	disc volatilization method	<i>Acinetobacter baumannii</i>	IZ 19 mm for 100 µL of EO	[99]
				<i>Bacillus subtilis</i>	IZ 30 mm for 100 µL of EO	[99]
				<i>Klebsiella pneumoniae</i>	MIC 20 mm for 100 µL of EO	[99]
				<i>Pseudomonas aeruginosa</i>	MIC 15 mm for 100 µL of EO	[99]
				<i>Staphylococcus aureus</i>	MIC 12–18 mm for 100 µL of EO	[99]

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference	
			multi-screening disc volatilization method	<i>Ascophæra apis</i>	MIC 500 µL/L	[80]	
<i>Lavandula latifolia</i>	Lamiaceae	flowery top	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 12.5 mg/L air	[53]	
				<i>Staphylococcus aureus</i>	MID 50 mg/L air	[53]	
				<i>Streptococcus pneumoniae</i>	MID 25 mg/L air	[53]	
				<i>Streptococcus pyogenes</i>	MID 25 mg/L air	[53]	
		not specified	multi-screening disc volatilization method	<i>Ascophæra apis</i>	MIC 500 µL/L	[80]	
<i>Lavandula vera</i>	Lamiaceae	not specified	disc volatilization method	<i>Candida albicans</i>	MIC 0.0019–0.0600 µg/mL	[110]	
				<i>Candida glabrata</i>	MIC 0.0075–0.0600 µg/mL	[110]	
				<i>Candida tropicalis</i>	MIC 0.015–0.060 µg/mL	[110]	
<i>Lawsonia inermis</i>	Lythraceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 23.3% 0.33 µL/mL	[81]	
				<i>Aspergillus niger</i>	O 32.4% 0.33 µL/mL	[81]	
				<i>Aspergillus ochraceus</i>	O 19.3% 0.33 µL/mL	[81]	
<i>Ledum groenlandicum</i>	Ericaceae	flower top	disc volatilization method	<i>Listeria monocytogenes</i>	IZ 27.9 mm for 30 µL of EO	[94]	
				<i>Staphylococcus aureus</i>	IZ 54.0 mm for 30 µL of EO	[94]	
<i>Limnophila aromatica</i>	Plantaginaceae	aerial parts	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 256 µg/mL	[87]	
<i>Lippia berlandieri</i>	Verbenaceae	not specified	disc volatilization method	<i>Aspergillus fumigatus</i>	MIC 0.50 µg/mL	[92]	
				<i>Aspergillus niger</i>	MIC 0.28 µg/mL	[92]	
				<i>Aspergillus nomius</i>	MIC 1.00 µg/mL	[92]	
				<i>Candida albicans</i>	MIC 0.25 µg/mL	[92]	
				<i>Cryptococcus neoformans</i>	MIC 0.25 µg/mL	[92]	
				<i>Escherichia coli</i>	MIC 4.0 µg/mL	[92]	
				<i>Eupenicillium hirayamae</i>	MIC 0.25 µg/mL	[92]	
				<i>Mycobacterium smegmatis</i>	MIC 3.5 µg/mL	[92]	
				<i>Penicillium cinnamopurpureum</i>	MIC 0.25 µg/mL	[92]	
				<i>Penicillium expansum</i>	MIC 0.26 µg/mL	[92]	
				<i>Penicillium viridicatum</i>	MIC 1.00 µg/mL	[92]	
<i>Trichophyton rubrum</i>	MIC 0.50 µg/mL	[92]					
<i>Litsea cubeba</i>	Lauraceae	fruit	airtight apparatus disc volatilization method	<i>Penicillium corylophilum</i>	MIC 0.1563 µL/L	[84]	
<i>Melaleuca alternifolia</i>	Myrtaceae	leaf	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 2.50 µL/mL	[63]	
				airtight box disc volatilization method	<i>Escherichia coli</i>	MID 50 mg/L air	[53]
					<i>Haemophilus influenzae</i>	MID 25 mg/L air	[53]
					<i>Staphylococcus aureus</i>	MID 50 mg/L	[53]
		disc volatilization method	<i>Staphylococcus aureus</i>	IZ 10.0 mm for 20 µL of EO	[59]		
		leaf, terminal branch	disc volatilization method	<i>Acinetobacter baumannii</i>	IZ 19.2 mm for 10 µL of EO	[112]	
				<i>Escherichia coli</i>	IZ 6.9 mm for 10 µL of EO	[112]	
				<i>Klebsiella pneumoniae</i>	IZ 5.0–5.6 mm for 10 µL of EO	[112]	
not specified	airtight box disc volatilization method	<i>Streptococcus pneumoniae</i>	MID 50 mg/L air	[53]			
		<i>Streptococcus pyogenes</i>	MID 50 mg/L air	[53]			

Continued

► **Table 1** *Continued*

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
			disc volatilization method	<i>Acinetobacter baumannii</i>	IZ 89 mm for 100 µL of EO	[99]
				<i>Bacillus subtilis</i>	IZ 18 mm for 100 µL of EO	[99]
				<i>Escherichia coli</i>	IZ 15.5–65.0 mm for 15–100 µL of EO	[99, 107]
				<i>Klebsiella pneumoniae</i>	IZ 13.0–22.5 mm for 15–100 µL of EO	[99, 107]
				<i>Pseudomonas aeruginosa</i>	IZ 90 mm for 100 µL of EO	[99]
				<i>Salmonella enteritidis</i>	IZ 17.5 mm for 15 µL of EO	[107]
				<i>Staphylococcus aureus</i>	IZ 12.0–25.0 mm for 15–20 µL of EO	[59, 107]
			<i>Staphylococcus epidermidis</i>	IZ 18.0 mm for 15 µL of EO	[107]	
			multi-screening disc volatilization method	<i>Salmonella enteritidis</i>	MIC 500 µL/L	[55]
<i>Melaleuca quinquenervia</i>	Myrtaceae	leaf	disc volatilization method	<i>Listeria monocytogenes</i>	IZ 26.8 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 29.8 mm for 30 µL of EO	[94]
<i>Melissa officinalis</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]
		not specified	disc volatilization method	<i>Candida albicans</i>	MIC 0.015–0.030 µg/mL	[110]
				<i>Candida glabrata</i>	MIC 0.015–0.060 µg/mL	[110]
				<i>Candida tropicalis</i>	MIC 0.0038–0.0150 µg/mL	[110]
<i>Mentha arvensis</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 250 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 125 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 250 µL/L	[55]
<i>Mentha × piperita</i>	Lamiaceae	aerial parts	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 0.625 µL/mL	[63]
			disc volatilization method	<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]
			not specified	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 12.5 mg/L air
		<i>Staphylococcus aureus</i>			MID 25 mg/L air	[53]
		<i>Streptococcus pneumoniae</i>			MID 25 mg/L air	[53]
		<i>Streptococcus pyogenes</i>			MID 25 mg/L air	[53]
		disc volatilization method	<i>Aspergillus flavus</i>	IZ 40.0–90.0 mm for 20–60.0 µL of EO	[113]	
			<i>Aspergillus niger</i>	IZ 43.0–90.0 mm for 20–60 µL of EO	[113]	
			<i>Bacillus subtilis</i>	IZ 27.0–46.0 mm for 20–60 µL of EO	[113]	
			<i>Candida albicans</i>	IZ 18.0–9.0 mm for 20–60 µL of EO	[106, 113]	
			<i>Colletotrichum gloeosporioides</i>	IZ 18.0–36.0 mm for 20–60 µL of EO; O 32.4–73.8% 1–5 µL EO per PD	[108, 113]	
		<i>Fusarium oxysporum</i>	IZ 60–90 mm for 20–60 µL of EO	[113]		

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
				<i>Lasiodiplodia theobromae</i>	O 18.9–69.3% 1–5 µL EO per PD	[108]
				<i>Monilinia fructicola</i>	O 56.9–80.5% 1–5 µL EO per PD	[108]
				<i>Mucor</i> spp.	IZ 52.0–90.0 mm for 20–60 µL of EO	[113]
				<i>Penicillium expansum</i>	O 26.2–69.2% 1–5 µL EO per PD	[108]
				<i>Pseudomonas aeruginosa</i>	IZ 18.0–28.0 mm for 20–60 µL of EO	[113]
				<i>Pseudomonas fluorescens</i>	IZ 16.0–28.0 mm for 20–60 µL of EO	[113]
				<i>Rhizopus stolonifer</i>	O 37.5–86.6% 1–5 µL EO per PD	[108]
<i>Mentha pulegium</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Staphylococcus aureus</i>	MIC 375 µL/L	[98]
				<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophaera apis</i>	MIC 500 µL/L	[80]
<i>Mentha spicata</i>	Lamiaceae	aerial parts	airtight apparatus disc volatilization method	<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
				<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]
		flowering herb	airtight apparatus disc volatilization method	<i>Penicillium corylophilum</i>	MIC 0.6250 µL/L	[84]
				not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>
<i>Ascophaera apis</i>	MIC 500 µL/L	[80]				
<i>Aspergillus niger</i>	MIC 500 µL/L	[55]				
<i>Mentha × villosa</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Penicillium digitatum</i>	MIC 500 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]
<i>Mentha</i> sp.	Lamiaceae	not specified	disc volatilization method	<i>Aureobasidium pullulans</i>	IZ 68.3–83.3 mm for 10–30 µL of EO	[114]
				<i>Candida diversa</i>	IZ 35.0–83.3 mm for 10–30 µL of EO	[114]
				<i>Hansenula polymorpha</i>	IZ 26.7–61.7 mm for 10–30 µL of EO	[114]
				<i>Pichia anomala</i>	IZ 29.2–51.7 mm for 10–30 µL of EO	[114]
				<i>Pichia fermentans</i>	IZ 10.0–26.7 mm for 10–30 µL of EO	[114]
				<i>Pichia kluyveri</i>	IZ 52.5–83.3 mm for 10–30 µL of EO	[114]
				<i>Saccharomyces cerevisiae</i>	IZ 38.3–83.3 mm for 10–30 µL of EO	[114]
				<i>Zygosaccharomyces bailii</i>	IZ 35.0–83.3 mm for 10–30 µL of EO	[114]

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Monarda didyma</i>	Lamiaceae	flowery top	disc volatilization method	<i>Escherichia coli</i>	IZ 45.6 mm for 30 µL of EO	[94]
				<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Pseudomonas aeruginosa</i>	IZ 28.1 mm for 30 µL of EO	[94]
				<i>Salmonella typhimurium</i>	IZ 40.3 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]
<i>Monarda fistulosa</i>	Lamiaceae	flowery top	disc volatilization method	<i>Escherichia coli</i>	IZ 20.1 mm for 30 µL of EO	[94]
				<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]
<i>Murraya paniculata</i>	Rutaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 37.3% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 29.8% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 45.0% 0.33 µL/mL	[81]
<i>Nepeta cataria</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 250 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Nepeta × faassenii</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]
<i>Nepeta hindostana</i>	Lamiaceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 38.7% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 34.3% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 42.4% 0.33 µL/mL	[81]
<i>Ocimum basilicum</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]
		not specified	disc volatilization method	<i>Peicillium citrinum</i>	IZ 1.00–52.83 mm for 50 µL of EO	[111]
				<i>Penicillium crustosum</i>	IZ 7.00–47.67 mm for 50 µL of EO	[111]
				<i>Penicillium expansum</i>	IZ 10.60–26.33 mm for 50 µL of EO	[111]
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 500 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
<i>Ocimum citriodorum</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 500 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 125 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 250 µL/L	[55]
<i>Ocimum gratissimum</i>	Lamiaceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 70.6% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 68.2% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 74.8% 0.33 µL/mL	[81]
<i>Origanum compactum</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 31.25 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 125 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 125 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 125 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 125 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 125 µL/L	[55]

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference	
<i>Origanum majorana</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	MIC 260 µL/L	[54]	
				<i>Staphylococcus aureus</i>	MIC 8.3–130.0 µL/L	[83]	
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]	
				<i>Ascophæra apis</i>	MIC 500 µL/L	[80]	
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]	
<i>Origanum syriacum</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 530 µL/L	[54]	
<i>Origanum vulgare</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	MIC 66 µL/L; IZ 36.1 mm for 30 µL of EO	[54, 95]	
				<i>Listeria monocytogenes</i>	MIC 66 µL/L; IZ 46.0 mm for 30 µL of EO	[54, 95]	
				<i>Salmonella enteritidis</i>	MIC 13 µL/L	[54]	
				<i>Staphylococcus aureus</i>	MIC 17 µL/L; IZ 78.0 mm for 30 µL of EO	[54, 95]	
		flower, leaf	disc volatilization method	<i>Bacillus cereus</i>	IZ 16.0 mm for 10 µL of EO	[104]	
				<i>Escherichia coli</i>	IZ 9.0 mm for 10 µL of EO	[104]	
				<i>Listeria monocytogenes</i>	IZ 15.0 mm for 10 µL of EO	[104]	
				<i>Salmonella typhimurium</i>	IZ 8.0–15.7 mm for 10–30 µL of EO	[95, 104]	
				<i>Staphylococcus aureus</i>	IZ 26.0 mm for 10 µL of EO	[104]	
		leaf	airtight apparatus disc volatilization method	<i>Penicillium corylophilum</i>	MIC 0.6250 µL/L	[84]	
		not specified	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 0.3125 µL/mL	[63]	
				disc volatilization method	<i>Escherichia coli</i>	MIC 64 µL/L	[96]
					<i>Salmonella typhimurium</i>	IZ 7.5–27.5 mm for 10 µL of EO	[94]
			<i>Salmonella enteritidis</i>		MIC 64 µL/L	[96]	
			multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 62.5–64.0 µL/L	[55, 97]	
				<i>Ascophæra apis</i>	MIC 63 µL/L	[80]	
				<i>Aspergillus niger</i>	MIC 62.5–128.0 µL/L	[53, 97]	
				<i>Botrytis cinerea</i>	MIC 32.0 µL/L	[97]	
				<i>Cladosporium cucumerinum</i>	MIC 64.0 µL/L	[97]	
				<i>Claviceps purpurea</i>	MIC 16.0 µL/L	[97]	
				<i>Dendryphion penicillatum</i>	MIC 128.0 µL/L	[97]	
<i>Helminthosporium solani</i>	MIC 32.0 µL/L			[97]			
<i>Monilia fructigena</i>	MIC 32.0 µL/L	[97]					
not specified	disc volatilization method	<i>Bacillus subtilis</i>	IZ 40 mm for 100 µL of EO	[99]			
		<i>Staphylococcus aureus</i>	IZ 48 mm for 100 µL of EO	[99]			

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Pelargonium graveolens</i>	Geraniaceae	aerial parts	disc volatilization method	<i>Bacillus cereus</i>	IZ 10.50–18.83 mm for 10–30 µL of EO	[115]
				<i>Bacillus subtilis</i>	IZ 22.83–50.83 mm for 10–30 µL of EO	[115]
				<i>Citrobacter freundii</i>	IZ 14.83–29.50 mm for 10–30 µL of EO	[115]
				<i>Enterobacter aerogenes</i>	IZ 7.0 mm for 30 µL of EO	[115]
				<i>Enterococcus faecalis</i>	IZ 22.33–54.50 mm for 10–30 µL of EO	[115]
				<i>Escherichia coli</i>	IZ 7.50–23.33 mm for 10–30 µL of EO	[115]
				<i>Staphylococcus aureus</i>	IZ 20.50–61.17 mm for 10–30 µL of EO	[115]
				<i>Staphylococcus epidermidis</i>	IZ 15.50–31.83 mm for 10–30 µL of EO	[115]
		not specified	disc volatilization method	<i>Escherichia coli</i>	IZ 14.5 mm for 15 µL of EO	[107]
				<i>Salmonella enteritidis</i>	IZ 6.0 mm for 15 µL of EO	[107]
				<i>Staphylococcus aureus</i>	IZ 10.0–25.0 mm for 15–20 µL of EO	[59, 107]
				<i>Staphylococcus epidermidis</i>	IZ 12.0 mm for 15 µL of EO	[107]
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 500 µL/L	[80]
<i>Aspergillus niger</i>	MIC 500 µL/L			[55]		
<i>Staphylococcus aureus</i>	MIC 500 µL/L			[55]		
<i>Pelargonium roseum</i>	Geraniaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Perilla</i> sp.	Lamiaceae	not specified	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 12.5 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 50 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 12.5–25.0 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 12.5 mg/L air	[53]
<i>Pimenta dioica</i>	Myrtaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 125–250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Pimenta racemosa</i>	Myrtaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 250 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 500 µL/L	[55]
<i>Pimpinella anisum</i>	Apiaceae	not specified	disc volatilization method	<i>Colletotrichum gloeosporioides</i>	O 3.5–52.1% 1–8 µL EO per PD	[52]
				<i>Lasiodiplodia theobromae</i>	O 2.0–52.7% 1–8 µL EO per PD	[52]

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Pinus sylvestris</i>	Pinaceae	leaf	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 5.0 µL/mL	[63]
		not specified	disc volatilization method	<i>Candida albicans</i>	MIC 0.5–1.0 µg/mL	[110]
				<i>Candida glabrata</i>	MIC 0.5–1.0 µg/mL	[110]
				<i>Candida tropicalis</i>	MIC 0.5–1.0 µg/mL	[110]
<i>Piper betle</i>	Piperaceae	leaf	disc volatilization method	<i>Penicillium expansum</i>	IZ 28.7 mm for 10 µL of EO	[116]
<i>Piper longum</i>	Piperaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 17.7% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 48.6% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 23.4% 0.33 µL/mL	[81]
<i>Piper nigrum</i>	Piperaceae	seed	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 2.50 µL/mL	[63]
<i>Pogostemon cablin</i>	Lamiaceae	not specified	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 17.5 mm for 20 µL of EO	[59]
			multi-screening disc volatilization method	<i>Ascophaera apis</i>	MIC 500 µL/L	[80]
				<i>Staphylococcus aureus</i>	MIC 500 µL/L	[55]
<i>Polyalthia longifolia</i>	Annonaceae	leaf	disc volatilization method	<i>Aspergillus flavus</i>	O 26.8% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 34.7% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 38.8% 0.33 µL/mL	[81]
<i>Pulicaria mauritanica</i>	Asteraceae	aerial parts	disc volatilization method	<i>Alternaria</i> sp.	MIC 0.125 µL/L	[117]
				<i>Aspergillus brasiliensis</i>	MIC 0.125 µL/L	[117]
				<i>Bacillus subtilis</i>	IZ 18.75–33.00 mm for 5–15 µL of EO	[117]
				<i>Fusarium oxysporum</i>	MIC 0.125 µL/L	[117]
				<i>Penicillium expansum</i>	MIC 0.25 µL/L	[117]
				<i>Rhizopus stolonifer</i>	MIC 0.125 µL/L	[117]
				<i>Salmonella abony</i>	IZ 14.33–26.00 mm for 10–15 µL of EO	[117]
<i>Rhodamnia dumetorum</i>	Myrtaceae	leaf	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 256 µg/mL	[87]
<i>Rosmarinus officinalis</i>	Lamiaceae	flower top	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 50 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 100 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 50 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 50 mg/L air	[53]
	not specified	disc volatilization method	<i>Penicillium citrinum</i>	IZ 1.50–38.33 mm for 50 µL of EO	[111]	
			<i>Penicillium crustosum</i>	IZ 6.50–47.50 mm for 50 µL of EO	[111]	
<i>Penicillium expansum</i>			IZ 11.17–29.17 mm for 50 µL of EO	[111]		
<i>Salvia lavandulifolia</i>	Lamiaceae	leaf and stem	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]
<i>Salvia officinalis</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 28.6 mm for 30 µL of EO	[94]
		not specified	disc volatilization method	<i>Candida albicans</i>	MIC 0.06 µg/mL	[110]
				<i>Candida glabrata</i>	MIC 0.125–0.250 µg/mL	[110]
				<i>Candida tropicalis</i>	MIC 0.06 µg/mL	[110]

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference	
<i>Salvia sclarea</i>	Lamiaceae	aerial parts	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]	
		not specified	multi-screening disc volatilization method	<i>Ascophaea apis</i>	MIC 500 µL/L	[80]	
<i>Satureja hortensis</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Staphylococcus aureus</i>	MIC 17.0–130.0 µL/L	[83]	
		flower top	disc volatilization method	<i>Escherichia coli</i>	IZ 42.8 mm for 30 µL of EO	[94]	
				<i>Listeria monocytogenes</i>	IZ 59.2 mm for 30 µL of EO	[94]	
				<i>Salmonella typhimurium</i>	IZ 22.9 mm for 30 µL of EO	[94]	
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]	
<i>Satureja montana</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	MIC 33 µL/L	[54]	
				<i>Listeria monocytogenes</i>	MIC 260 µL/L	[54]	
				<i>Salmonella enteritidis</i>	MIC 260 µL/L	[54]	
				<i>Staphylococcus aureus</i>	MIC 17.0–130.0 µL/L	[54, 83]	
<i>Sindora siamensis</i>	Leguminosae	fruit husk	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 64 µg/mL	[87]	
<i>Solidago canadensis</i>	Asteraceae	flowery top	disc volatilization method	<i>Listeria monocytogenes</i>	IZ 36.1 mm for 30 µL of EO	[94]	
				<i>Staphylococcus aureus</i>	IZ 30.1 mm for 30 µL of EO	[94]	
<i>Syzygium aromaticum</i>	Myrtaceae	floral bud	disc volatilization method	<i>Escherichia coli</i>	MIC 256 µL/L; IZ 28.1 mm for 30 µL of EO	[95, 96]	
				<i>Listeria monocytogenes</i>	IZ 26.8 mm for 30 µL of EO	[94]	
				<i>Salmonella enteritidis</i>	MIC 512 µL/L	[96]	
				<i>Salmonella typhimurium</i>	IZ 20.7 mm for 30 µL of EO	[94]	
				<i>Staphylococcus aureus</i>	IZ 42.4 mm for 30 µL of EO	[94]	
				multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 64 µL/L	[97]
					<i>Aspergillus niger</i>	MIC 256 µL/L	[97]
					<i>Botrytis cinerea</i>	MIC 64 µL/L	[97]
					<i>Cladosporium cucumerinum</i>	MIC 128 µL/L	[97]
					<i>Claviceps purpurea</i>	MIC 32 µL/L	[97]
					<i>Dendryphion penicillatum</i>	MIC 128 µL/L	[97]
					<i>Helminthosporium solani</i>	MIC 256 µL/L	[97]
				<i>Monilia fructigena</i>	MIC 256 µL/L	[97]	
		<i>Penicillium digitatum</i>	MIC 256 µL/L	[97]			
		<i>Penicillium expansum</i>	MIC 256 µL/L	[97]			
		<i>Phoma foveata</i>	MIC 256 µL/L	[97]			
		leaf and stem	disc volatilization method	<i>Escherichia coli</i>	MIC 26.2 µL/L	[57]	
		not specified	airtight apparatus disc volatilization method	disc volatilization method	<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]
					<i>Aspergillus flavus</i>	MIC 17.5 µL/L	[57]
					<i>Bacillus cereus</i>	IZ 21 mm for 10 µL of EO	[60]
<i>Bacillus cereus</i>	MIC 17.5 µL/L				[57]		
<i>Candida albicans</i>	MIC 13.1 µL/L; 0.25–1.00 µg/mL				[57, 110]		
<i>Candida glabrata</i>	MIC 0.25–0.50 µg/mL				[110]		

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
				<i>Candida tropicalis</i>	MIC 0.06–1.00 µg/mL	[110]
				<i>Colletotrichum gloeosporioides</i>	O 36.2–100.0% 1–8 µL EO per PD	[52]
				<i>Enterococcus faecalis</i>	MIC 87.3 µL/L; IZ 12 mm for 10 µL of EO	[57, 60]
				<i>Escherichia coli</i>	IZ 30 mm for 10 µL of EO	[60]
				<i>Lasiodiplodia theobromae</i>	O 42.7–100.0% 1–8 µL EO per PD	[52]
				<i>Listeria monocytogenes</i>	MIC 17.5 µL/L; IZ 13 mm for 10 µL of EO	[56, 59]
				<i>Penicillium islandicum</i>	MIC 8.73 µL/L	[57]
				<i>Salmonella choleraesuis</i>	MIC 52.4 µL/L; IZ 13 mm for 10 µL of EO	[57, 60]
				<i>Staphylococcus aureus</i>	MIC 26.2 µL/L; IZ 23 mm for 10 µL of EO	[57, 60]
			<i>Yersinia enterocolitica</i>	MIC 8.73 µL/L; IZ 35 mm for 10 µL of EO	[57, 60]	
			multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 500 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 500 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 225–250 µL/L	[55, 98]
<i>Thuja occidentalis</i>	Cupressaceae	branch	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 27.6 mm for 30 µL of EO	[94]
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 500 µL/L	[80]
<i>Thymus capitatus</i>	Lamiaceae	not specified	disc volatilization method	<i>Bacillus cereus</i>	IZ 41.6 mm for 10 µL of EO	[82]
				<i>Escherichia coli</i>	IZ 27.0 mm for 10 µL of EO	[82]
				<i>Salmonella enteritidis</i>	IZ 43.0 mm for 10 µL of EO	[82]
				<i>Staphylococcus aureus</i>	IZ 26.3 mm for 10 µL of EO	[82]
<i>Thymus mastichina</i>	Lamiaceae	leaf and stem	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 1.25 µL/mL	[63]
		not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 500 µL/L	[55]
<i>Thymus pulegioides</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Listeria monocytogenes</i>	MIC 260 µL/L	[54]
				<i>Staphylococcus aureus</i>	MIC 33 µL/L	[54]
				<i>Escherichia coli</i>	MIC 33 µL/L	[54]
				<i>Salmonella enteritidis</i>	MIC 260 µL/L	[54]
<i>Thymus saturooides</i>	Lamiaceae	not specified	multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125 µL/L	[55]
				<i>Ascophæra apis</i>	MIC 125 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]
				<i>Salmonella enteritidis</i>	MIC 125 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 125 µL/L	[55]

Continued

► **Table 1** *Continued*

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
<i>Thymus serpyllum</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	MIC 33 µL/L	[54]
				<i>Staphylococcus aureus</i>	MIC 33–260 µL/L	[54, 83]
				<i>Streptococcus pneumoniae</i>	MIC 33 µL/L	[54]
		not specified	airtight box disc volatilization method	<i>Escherichia coli</i>	MID 12.5 mg/L air	[53]
				<i>Haemophilus influenzae</i>	MID 3.13 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 12.5 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 3.13 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 6.25 mg/L air	[53]
		disc volatilization method	<i>Acinetobacter baumannii</i>	IZ 18–29 mm for 100 µL of EO	[99]	
			<i>Bacillus subtilis</i>	IZ 29 mm for 100 µL of EO	[99]	
			<i>Escherichia coli</i>	IZ 29 mm for 100 µL of EO	[99]	
			<i>Listeria monocytogenes</i>	530 µL/L	[54]	
			<i>Pseudomonas aeruginosa</i>	IZ 55 mm for 100 µL of EO	[99]	
			<i>Staphylococcus aureus</i>	IZ 29–35 mm for 100 µL of EO	[99]	
		multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 250 µL/L	[55]	
			<i>Ascophaea apis</i>	MIC 250 µL/L	[80]	
			<i>Aspergillus niger</i>	MIC 250 µL/L	[55]	
			<i>Penicillium digitatum</i>	MIC 250 µL/L	[55]	
			<i>Salmonella enteritidis</i>	MIC 250 µL/L	[55]	
			<i>Staphylococcus aureus</i>	MIC 250 µL/L	[55]	
<i>Thymus vulgaris</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	IZ 34.6 mm for 30 µL of EO	[94]
				<i>Escherichia coli</i>	MIC 33 µL/L	[54]
				<i>Listeria monocytogenes</i>	IZ 37.2 mm for 30 µL of EO	[94]
				<i>Salmonella enteritidis</i>	MIC 33 µL/L	[54]
				<i>Salmonella typhimurium</i>	IZ 15.2 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	MIC 17–260 µL/L; IZ 78 mm for 30 µL of EO	[54, 83, 95]
		flower, leaf	disc volatilization method	<i>Bacillus cereus</i>	IZ 12.0 mm for 10 µL of EO	[104]
				<i>Escherichia coli</i>	IZ 15.0 mm for 10 µL of EO	[104]
				<i>Listeria monocytogenes</i>	IZ 51.46–67.50 mm for 0.18–0.72 µL/mL EO	[118]
				<i>Salmonella typhimurium</i>	IZ 11.0 mm for 10 µL of EO	[104]
				<i>Staphylococcus aureus</i>	IZ 24.0 mm for 10 µL of EO	[104]
		flowery top	airtight box disc volatilization method	<i>Haemophilus influenzae</i>	MID 12.5 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 50 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 6.25 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 12.5 mg/L air	[53]
		not specified	airtight box disc volatilization method	<i>Escherichia coli</i>	MID 12.5 mg/L air	[53]
				<i>Haemophilus influenzae</i>	MID 3.13 mg/L air	[53]
				<i>Staphylococcus aureus</i>	MID 6.25 mg/L air	[53]
				<i>Streptococcus pneumoniae</i>	MID 6.25 mg/L air	[53]
				<i>Streptococcus pyogenes</i>	MID 3.13 mg/L air	[53]
disc volatilization method	<i>Acinetobacter baumannii</i>		IZ 40–50 mm for 100 µL of EO	[99]		
	<i>Aspergillus fumigatus</i>		MIC 0.50 µg/mL	[92]		
	<i>Aspergillus niger</i>		MIC 0.20 µg/mL	[92]		

Continued

► Table 1 Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
				<i>Aspergillus nomius</i>	MIC 0.50 µg/mL	[92]
				<i>Bacillus cereus</i>	IZ 32.0 mm for 10 µL of EO	[82]
				<i>Bacillus subtilis</i>	IZ 38 mm for 100 µL of EO	[99]
				<i>Candida albicans</i>	MIC < 0.0038–0.1000 µg/mL	[92, 110]
				<i>Candida glabrata</i>	MIC < 0.0019–0.0300 µg/mL	[110]
				<i>Candida tropicalis</i>	MIC 0.0075–0.0100 µg/mL	[110]
				<i>Colletotrichum gloeosporioides</i>	O 64.9–100.0% 1–8 µL EO per PD	[52, 108]
				<i>Cryptococcus neoformans</i>	MIC 0.25 µg/mL	[92]
				<i>Escherichia coli</i>	MIC 10 mg/L; 4.0 µg/mL; IZ 46.3–85.0 mm for 10–100 µL of EO	[82, 91, 99, 100]
				<i>Eupenicillium hirayamae</i>	MIC 0.25 µg/mL	[92]
				<i>Klebsiella pneumoniae</i>	IZ 33 mm for 100 µL of EO	[99]
				<i>Lasiodiplodia theobromae</i>	O 71.1–100.0% 1–8 µL EO per PD	[52, 108]
				<i>Listeria monocytogenes</i>	MIC 260 µL/L	[54]
				<i>Monilinia fructicola</i>	O 69.1–100.0% 1–5 µL EO per PD	[108]
				<i>Mycobacterium smegmatis</i>	MIC 3.5 µg/mL	[92]
				<i>Penicillium cinnamopurpureum</i>	MIC 0.25 µg/mL	[92]
				<i>Penicillium expansum</i>	MIC 0.16 µg/mL; O 68.7– 100.0% 1–5 µL EO per PD	[92, 108]
				<i>Penicillium viridicatum</i>	MIC 0.50 µg/mL	[92]
				<i>Pseudomonas aeruginosa</i>	IZ 85 mm for 100 µL of EO	[99]
				<i>Rhizopus stolonifer</i>	O 62.2–100.0% 1–5 µL EO per PD	[108]
				<i>Salmonella enteritidis</i>	IZ 48.3 mm for 10 µL of EO	[82]
				<i>Staphylococcus aureus</i>	MIC 20 mg/L; IZ 37.0– 52.0 mm for 10–100 µL of EO	[82, 99, 100]
				<i>Trichophyton rubrum</i>	MIC 0.50 µg/mL	[92]
			multi-screening disc volatilization method	<i>Alternaria alternata</i>	MIC 125–250 µL/L	[55]
				<i>Ascophaea apis</i>	MIC 31–250 µL/L	[80]
				<i>Aspergillus niger</i>	MIC 125–250 µL/L	[55]
				<i>Penicillium digitatum</i>	MIC 125–500 µL/L	[55]
				<i>Pseudomonas aeruginosa</i>	MIC 31.25 µL/L	[98]
				<i>Salmonella enteritidis</i>	MIC 125 µL/L	[55]
				<i>Staphylococcus aureus</i>	MIC 125–250 µL/L	[55, 98]
<i>Thymus zygis</i>	Lamiaceae	aerial parts	airtight apparatus disc volatilization method	<i>Escherichia coli</i>	MIC 0.0781–0.6250 µL/mL	[63]
			disc volatilization method	<i>Escherichia coli</i>	IZ 47.0 mm for 30 µL of EO	[94]
				<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Salmonella typhimurium</i>	IZ 25.4 mm for 30 µL of EO	[94]
				<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]

Continued

► **Table 1** Continued

Plant species	Plant families	Part of the plant used	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
		leaf	agar plug-based vapor phase assay	<i>Histophilus somni</i>	O complete inhibition	[50]
				<i>Mannheimia haemolytica</i>	O complete inhibition	[50]
				<i>Pasteurella multocida</i>	O complete inhibition	[50]
					airtight apparatus disc volatilization method	<i>Penicillium corylophilum</i>
<i>Trachyspermum ammi</i>	Apiaceae	seed	agar plug-based vapor phase assay	<i>Histophilus somni</i>	O complete inhibition	[50]
				<i>Mannheimia haemolytica</i>	O complete inhibition	[50]
				<i>Pasteurella multocida</i>	O complete inhibition	[50]
			disc volatilization method	<i>Escherichia coli</i>	IZ 26.4 mm for 30 µL of EO	[94]
				<i>Listeria monocytogenes</i>	IZ 78.0 mm for 30 µL of EO	[94]
				<i>Salmonella typhimurium</i>	IZ 26.6 mm for 30 µL of EO	[94]
		<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]		
<i>Tsuga canadensis</i>	Pinaceae	branch, needle	disc volatilization method	<i>Staphylococcus aureus</i>	IZ 78.0 mm for 30 µL of EO	[94]
<i>Vitex negundo</i>	Lamiaceae	shoot	disc volatilization method	<i>Aspergillus flavus</i>	O 16.6% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 21.4% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 19.2% 0.33 µL/mL	[81]
		not specified	disc volatilization method	<i>Colletotrichum gloeosporioides</i>	O 4.2–50.0% 1–8 µL EO per PD	[52]
				<i>Lasiodiplodia theobromae</i>	O 1.5–47.3% 1–8 µL EO per PD	[52]
<i>Wasabia japonica</i>	Brassicaceae	rhizome	disc volatilization method	<i>Aspergillus flavus</i>	MIC 1.5%	[51]
				<i>Aspergillus niger</i>	MIC 0.4%; IZ 20 mm	[51]
				<i>Aspergillus ochraceus</i>	MIC 0.4%	[51]
				<i>Penicillium lanosum</i>	MIC 0.2–0.8%; IZ 5–100 mm	[51]
				<i>Penicillium purpurogenum</i>	MIC 0.4%	[51]
				<i>Penicillium simplicissimum</i>	MIC 0.8%	[51]
				<i>Ulocladium</i> sp.	MIC 1.5%; IZ 8 mm	[51]
<i>Zataria multiflora</i>	Lamiaceae	aerial parts	disc volatilization method	<i>Escherichia coli</i>	MIC 25 µg	[119]
				<i>Listeria monocytogenes</i>	MIC 25 µg	[119]
		not specified	disc volatilization method	<i>Bacillus cereus</i>	IZ 2.51–23.18 mm	[120]
				<i>Escherichia coli</i>	IZ 10.43–22.35 mm	[120]
				<i>Pseudomonas aeruginosa</i>	IZ 8.09–10.67 mm	[120]
				<i>Salmonella typhimurium</i>	IZ 7.25–17.29 mm	[120]
				<i>Staphylococcus aureus</i>	IZ 11.69–20.16 mm	[120]
<i>Zingiber cassumunar</i>	Zingiberaceae	not specified	multi-screening disc volatilization method	<i>Ascophaea apis</i>	MIC 500 µL/L	[80]
<i>Zingiber officinale</i>	Zingiberaceae	rhizome	disc volatilization method	<i>Aspergillus flavus</i>	O 45.0% 0.33 µL/mL	[81]
				<i>Aspergillus niger</i>	O 55.4% 0.33 µL/mL	[81]
				<i>Aspergillus ochraceus</i>	O 22.2% 0.33 µL/mL	[81]

MIC: minimum inhibitory concentration, MID: minimum inhibitory dose; IZ: inhibition zone; O: others

► **Table 2** Antimicrobial activity of plant volatiles tested in vitro in the vapour phase.

Compound	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
Allyl isothiocyanate	disc volatilization method	<i>Salmonella typhimurium</i>	IZ 40.0–85.0 mm for 10 µL of compound	[94]
Benzalkonium chloride	disc volatilization method	<i>Aspergillus europaeus</i>	O 19.7–72.1 % 4–12 mg/cm ³	[91]
		<i>Aspergillus niger</i>	O 19.0–40.8 % 8–12 mg/cm ³	[91]
		<i>Cladosporium cladosporioides</i>	O 17.7–72.1 % 8–12 mg/cm ³	[91]
		<i>Cladosporium uredinicola</i>	O 42.2–63.9 % 4–12 mg/cm ³	[91]
		<i>Penicillium atosanguineum</i>	O 6.8–53.1 % 4–12 mg/cm ³	[91]
		<i>Penicillium bilaiae</i>	O 23.1–65.3 % 4–12 mg/cm ³	[91]
		<i>Penicillium lanosum</i>	O 29.9–76.2 % 4–12 mg/cm ³	[91]
Carvacrol	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 64 µg/mL	[65]
		<i>Staphylococcus aureus</i>	MIC 64–1024 µg/mL	[65, 66]
		<i>Streptococcus pneumoniae</i>	MIC 64 µg/mL	[65]
	disc volatilization method	<i>Aggregatibacter actinomycetemcomitans</i>	IZ 1.3 mm for 500 µg of compound	[121]
		<i>Bacillus subtilis</i>	MID 10 mg/petri dish	[122]
		<i>Borytis cinerea</i>	MID 5 mg/petri dish	[122]
		<i>Candida albicans</i>	MIC 0.0038 µg/mL	[110]
		<i>Candida glabrata</i>	MIC < 0.0019 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC < 0.0019–0.0038 µg/mL	[110]
		<i>Escherichia coli</i>	MIC < 5 mg/L; MID 5 mg/petri dish; IZ 1.3 mm for 500 µg of compound	[100, 121, 122]
		<i>Geotrichum candidum</i>	MIC 80 mg/L	[123]
		<i>Lactobacillus plantarus</i>	MID 20 mg/petri dish	[122]
		<i>Lasioidiplodia</i> spp.	MIC 40 mg/L	[123]
		<i>Pestalotiopsis</i> spp.	MIC 40 mg/L	[123]
		<i>Phomopsis</i> spp.	MIC 40 mg/L	[123]
		<i>Pseudomonas fluorescenc</i>	MID 10 mg/petri dish	[122]
		<i>Saccharomyces cerevisiae</i>	MID 5 mg/petri dish	[122]
		<i>Salmonella typhimurium</i>	IZ 13.75–62.50 mm for 10 µL of compound	[94]
		<i>Staphylococcus aureus</i>	MIC 10 mg/L; MID 5 mg/Petri dish	[100, 122]
		Cinnamaldehyde	broth microdilution volatilization	<i>Haemophilus influenzae</i>
<i>Staphylococcus aureus</i>	MIC 64 µg/mL			[65]
<i>Streptococcus pneumoniae</i>	MIC 64 µg/mL			[65]
disc volatilization method	<i>Escherichia coli</i>		MIC 10 mg/L	[100]
	<i>Salmonella typhimurium</i>		IZ 7.50–78.75 mm for 10 µL of compound	[94]
	<i>Staphylococcus aureus</i>		MIC 20 mg/L	[100]
Citral	disc volatilization method	<i>Bacillus cereus</i>	IZ > 90 mm	[101]
		<i>Enterococcus faecalis</i>	MID 400–1600 mg/L	[124]
		<i>Enterococcus faecium</i>	MID 400–800 mg/L	[124]
		<i>Listeria monocytogenes</i>	IZ 79 mm	[101]
		<i>Staphylococcus aureus</i>	IZ 47 mm	[101]
Citronellal	airtight apparatus liquid volatilization method	<i>Aspergillus candidus</i>	MID 28 mg/L air	[125]
		<i>Aspergillus flavus</i>	MID 56 mg/L air	[125]
		<i>Aspergillus versicolor</i>	MID 28 mg/L air	[125]
		<i>Eurotium amstelodami</i>	MID 28 mg/L air	[125]
		<i>Eurotium chevalieri</i>	MID 14 mg/L air	[125]

Continued

► **Table 2** *Continued*

Compound	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
		<i>Penicillium adametzii</i>	MID 56 mg/L air	[125]
		<i>Penicillium citrinum</i>	MID 28 mg/L air	[125]
		<i>Penicillium griseofulvum</i>	MID 56 mg/L air	[125]
		<i>Penicillium islandicum</i>	MID 14 mg/L air	[125]
Citronellol	disc volatilization method	<i>Salmonella enteritidis</i>	IZ 6.0 mm for 15 µL of compound	[107]
		<i>Staphylococcus aureus</i>	IZ 12.0 mm for 15 µL of compound	[107]
		<i>Staphylococcus epidermidis</i>	IZ 12.0 mm for 15 µL of compound	[107]
Eugenol	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 128 µg/mL	[65]
		<i>Staphylococcus aureus</i>	MIC 128 µg/mL	[65]
		<i>Streptococcus pneumoniae</i>	MIC 256 µg/mL	[65]
	disc volatilization method	<i>Bacillus subtilis</i>	MID 15 mg/Petri dish	[122]
		<i>Borytis cinerea</i>	MID 10 mg/Petri dish	[122]
		<i>Candida albicans</i>	MIC 0.125–0.500 µg/mL	[110]
		<i>Candida glabrata</i>	MIC 0.06–0.25 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC 0.125–0.250 µg/mL	[110]
		<i>Escherichia coli</i>	MID 10 mg/Petri dish	[122]
		<i>Mucor</i> sp.	IZ 9.0 mm for 2.44–4.88 µL/400 mL air	[126]
		<i>Rhizopus stolonifer</i>	IZ 9.0 mm for 2.44–4.88 µL/400 mL air	[126]
		<i>Saccharomyces cerevisiae</i>	MID 10 mg/petri dish	[122]
		<i>Sclerotinia sclerotiorum</i>	IZ 9.0 mm for 2.44–4.88 µL/400 mL air	[126]
		<i>Staphylococcus aureus</i>	MID 5 mg/petri dish	[122]
Geraniol	disc volatilization method	<i>Klebsiella pneumoniae</i>	IZ 8.5 mm for 15 µL of compound	[107]
		<i>Staphylococcus aureus</i>	IZ 12.5 mm for 15 µL of compound	[107]
		<i>Staphylococcus epidermidis</i>	IZ 9.0 mm for 15 µL of compound	[107]
Hinokitiol	disc volatilization method	<i>Aggregatibacter actinomycetemcomitans</i>	IZ 4.4 mm for 500 µg of compound	[121]
		<i>Escherichia coli</i>	IZ 4.3 mm for 500 µg of compound	[121]
		<i>Staphylococcus aureus</i>	IZ 3.9 mm for 500 µg of compound	[121]
		<i>Streptococcus mutans</i>	IZ 4.1 mm for 500 µg of sample	[121]
8-Hydroxyquinoline	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 4 µg/mL	[65]
		<i>Staphylococcus aureus</i>	MIC 2 µg/mL	[65]
		<i>Streptococcus pneumoniae</i>	MIC 32 µg/mL	[65]
Linalool	airtight apparatus liquid volatilization method	<i>Aspergillus candidus</i>	MID 28 mg/L air	[125]
		<i>Aspergillus flavus</i>	MID 56 mg/L air	[125]
		<i>Aspergillus versicolor</i>	MID 56 mg/L air	[125]
		<i>Eurotium amstelodami</i>	MID 28 mg/L air	[125]
		<i>Eurotium chevalieri</i>	MID 56 mg/L air	[125]
		<i>Penicillium adametzii</i>	MID 28 mg/L air	[125]
		<i>Penicillium citrinum</i>	MID 28 mg/L air	[125]
		<i>Penicillium griseofulvum</i>	MID 56 mg/L air	[125]
		<i>Penicillium islandicum</i>	MID 28 mg/L air	[125]
	disc volatilization method	<i>Bacillus cereus</i>	IZ 35 mm	[101]
		<i>Candida albicans</i>	MIC 0.0075 µg/mL	[110]
		<i>Candida glabrata</i>	MIC 0.0075–0.0300 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC 0.0015–0.0300 µg/mL	[110]
		<i>Enterococcus faecalis</i>	MID 100–1600 mg/L	[124]

Continued

► Table 2 Continued

Compound	Antimicrobial method	Microorganism	MIC, MID, IZ, O	Reference
		<i>Enterococcus faecium</i>	MID 100–200 mg/L	[124]
		<i>Listeria monocytogenes</i>	IZ 62 mm	[101]
		<i>Mucor</i> sp.	IZ 5.7–9.0 mm for 11.48–17.22 µL/400 mL air	[126]
		<i>Rhizopus stolonifer</i>	IZ 4.1–6.2 mm for 11.48–17.22 µL/400 mL air	[126]
		<i>Sclerotinia sclerotiorum</i>	IZ 2.1–9.0 mm for 11.48–22.96 µL/400 mL air	[126]
		<i>Staphylococcus aureus</i>	IZ > 90 mm	[101]
Linalyl acetate	disc volatilization method	<i>Candida albicans</i>	MIC 1 µg/mL	[110]
		<i>Candida glabrata</i>	MIC 1 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC 1 µg/mL	[110]
Menthol	disc volatilization method	<i>Bacillus subtilis</i>	MID 20 mg/petri dish	[122]
		<i>Escherichia coli</i>	MID 30 mg/petri dish	[122]
		<i>Mucor</i> sp.	IZ 2.6 mm for 5.95 µg/400 mL air	[126]
		<i>Rhizopus stolonifer</i>	IZ 4.4 mm for 5.95 µg/400 mL air	[126]
		<i>Saccharomyces cerevisiae</i>	MID 20 mg/petri dish	[122]
		<i>Sclerotinia sclerotiorum</i>	IZ 1.8 mm for 5.95 µg/400 mL air	[126]
		<i>Staphylococcus aureus</i>	MID 30 mg/petri dish	[122]
Menthone	disc volatilization method	<i>Mucor</i> sp.	IZ 7.3–9.0 mm for 7.4–14.8 µL/400 mL air	[126]
		<i>Rhizopus stolonifer</i>	IZ 9.0 mm for 7.4–14.8 µL/400 mL air	[126]
		<i>Sclerotinia sclerotiorum</i>	IZ 5.7–9.0 mm for 7.4–14.8 µL/400 mL air	[126]
α-Pinene	disc volatilization method	<i>Candida albicans</i>	MIC 0.25–0.50 µg/mL	[110]
		<i>Candida glabrata</i>	MIC 0.25–0.50 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC 0.25–1.00 µg/mL	[110]
Terpinen-4-ol	disc volatilization method	<i>Escherichia coli</i>	IZ 18.0 mm for 15 µL of sample	[107]
		<i>Klebsiella pneumoniae</i>	IZ 26.5 mm for 15 µL of sample	[107]
		<i>Salmonella enteritidis</i>	IZ 20.0 mm for 15 µL of sample	[107]
		<i>Staphylococcus aureus</i>	IZ 17.5 mm for 15 µL of sample	[107]
		<i>Staphylococcus epidermidis</i>	IZ 11.0 mm for 15 µL of sample	[107]
Thymol	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 32 µg/mL	[65]
		<i>Staphylococcus aureus</i>	MIC 32–1024 µg/mL	[65, 66]
		<i>Streptococcus pneumoniae</i>	MIC 32 µg/mL	[65]
	disc volatilization method	<i>Aggregatibacter actinomycetemcomitans</i>	IZ 1.9 mm for 500 µg of sample	[121]
		<i>Candida albicans</i>	MIC 0.0038 µg/mL	[110]
		<i>Candida glabrata</i>	MIC 0.0019–0.0038 µg/mL	[110]
		<i>Candida tropicalis</i>	MIC < 0.0019 µg/mL	[110]
		<i>Escherichia coli</i>	IZ 1.3 mm for 500 µg of sample	[121]
		<i>Geotrichum candidum</i>	MIC 80 mg/L	[123]
		<i>Lasioidiplodia</i> spp.	MIC 40 mg/L	[123]
		<i>Pestalotiopsis</i> spp.	MIC 40 mg/L	[123]
<i>Phomopsis</i> spp.	MIC 40 mg/L	[123]		
Thymoquinone	broth microdilution volatilization	<i>Haemophilus influenzae</i>	MIC 2 µg/mL	[65]
		<i>Staphylococcus aureus</i>	MIC 4 µg/mL	[65]
		<i>Streptococcus pneumoniae</i>	MIC 8 µg/mL	[65]

MIC: minimum inhibitory concentration; MID: minimum inhibitory dose; IZ: inhibition zone; O: others

hydrophobicity, and viscosity. Their hydrophobic nature worsens the solubility of these compounds in water-based media (e.g., agar, broth), which may reduce their capability for dilution and result in an unequal distribution of active components throughout the medium, as seen in the case of direct contact methods such as broth dilution and disc diffusion tests, even if a proper dispersing or solubilizing agent such as Tween 20/80 or Span 20/80 is used [38,39]. Volatility causes a risk of active substance losses via evaporation during sample handling, experiment preparation, and incubation, depending on time and temperature conditions [40,41]. Although, in conventional assays based on disc diffusion and dilution tests, this can be prevented by using vapour barriers such as an ethylene vinyl acetate (EVA) capmat [42] and a cover glass with a plastic seal ring [43], this approach does not solve the problem of the assessment of the antimicrobial potential of VAs in a vapour phase. In this case, the interaction of VAs with the matrix onto which they are applied (e.g., paper disc, cultivation broth) is a crucial aspect affecting the speed and intensity of their evaporation into the atmosphere. For example, less evaporation was observed when the compound was mixed into the broth [44]. Working in a chamber with a saturated moistened atmosphere or high-water activity levels could improve the situation and increase the effectivity of VAs [45]. Therefore, a carrier medium/matrix selection is a critical point in the practical use and suitability of *in vitro* assaying.

In contrast to well-established assay methods for the testing of antimicrobial susceptibility on solid (agar disc diffusion) and in liquid (broth microdilution) media, there are no standardized methods for the determination of microbial sensitivity to volatile compounds in the vapour phase, e.g., in accordance with the Clinical and Laboratory Standards Institute (CLSI) [46,47], the National Committee for Clinical Laboratory Standards (NCCLS) [48], or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [49]. In recent years, several methods for testing the antimicrobial effects of VAs have been developed with the aim of studying the potential of their vapours in inhibiting the growth of pathogenic microorganisms, most of which are modifications of a disc diffusion assay. Although these methods allow for the creation of relative values, it is quite difficult to determine accurate results for microbial inhibition, and their main limitation lies in providing only qualitative measurements [50]. In consequence of this, the results are expressed in different ways, such as the diameter of the inhibition zones, an inhibition ratio [51], a minimum inhibitory dose per colony-forming units [50], the percentage inhibition of radial microbial growth [52], a unit volume of air [53], and various definitions of minimum inhibitory concentration (MIC) [54–56], which complicate their comparability.

The main aim of this study was to review the methods developed for the evaluation of the growth-inhibitory effects of volatiles in a gaseous phase by systematically reviewing the available literature data published in 2000–2020. Papers published in 1983–1999 were used when necessary to explain some concepts and principles. The search was conducted in Web of Science and Scopus databases based on the following key words: antibacterial; broth dilution; diffusion; essential oils; *in vitro* methods, plant volatiles; vapour phase, and volatilization. The reviewed techniques were newly categorized into two groups: solid and liquid matrix

volatilization methods, depending on whether the tested volatiles were applied to the solid matrices (e.g., paper disc) or were in liquid form (e.g., pure compounds or dissolved in broth or solvents). For the purposes of this classification, the names of some methods were modified, however, the original names are included in text as well. In addition, the antimicrobial efficacy of vapours examined using different *in vitro* tests against a broad spectrum of microorganisms is summarized.

Solid matrix volatilization methods

Disc volatilization assay

This assay method (also known as the inverted petri plate method) based on a very simple modification of the standardized disc diffusion method is the most frequently used to evaluate the antimicrobial effects of volatiles in the vapour phase. Petri dishes containing the appropriate solidified medium are inoculated with the solution containing the microorganism to be tested. Then, sterile filter paper discs are impregnated with the volatile compound at a desired concentration and put on the medium-free cover of the petri dishes. The plates are immediately inverted on top of the lid and sealed with parafilm or sterile adhesive tape to prevent any leakage of vapours of the active component to the atmosphere. The petri dishes are incubated under suitable conditions according to the microbial pathogens tested. Generally, antimicrobial agents diffuse from the disc to the atmosphere inside the petri dishes and then to the agar, which inhibits the growth of the test microorganism [57]. The diameters of inhibition zones are regarded as a measure of their antimicrobial activity, which can be interpreted according to the following criteria: weak activity (inhibition zone ≤ 12 mm), moderate activity ($12 \text{ mm} < \text{inhibition zone} < 20 \text{ mm}$), and strong activity (inhibition zone $\leq 20 \text{ mm}$) [44].

With the aim of improving the reliability and usability of the disc volatilization assay method, various modifications have been suggested (► Fig. 1). Although methods based on the disc volatilization assay method performed in petri dishes are a useful tool in the simple and low-cost assessment of the growth-inhibitory potential of EOs in the vapour phase, they are not designed for high-throughput screening. The relatively high consumption of material and labour are the main disadvantages in most of them, because each concentration of each VA must be tested on a separate disc. Moreover, the disc volatilization method as an example of qualitative assaying is not appropriate for the determination of MICs because it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium. Nevertheless, an approximate MIC can be calculated as microlitres of VA per volume unit of atmosphere above the organism growing on the agar surface that caused apparent inhibition, as in the study by Lopez et al. [57]. The results observed by these methods have been found to vary significantly because they are influenced by several parameters such as disc size (diameter ranges 3–10 mm), the amount of compound applied on the disc (volume varying from 10 to 260 μL), and the type of agar and its volume [44].

Nedorostova et al. [54] modified the disc volatilization method by using agar sealing with a warm medium poured into a petri dish and its cover. The solidified medium in the dish part is inoculated with the microorganism to be tested, while the agar in the lid serves as a sealing and prevents the adsorption of EOs into the

plastic material of the petri dish cover. As another option is, when the microorganisms are seeded on both agar parts; this test combines the principles of standard disc diffusion and disc volatilization assay methods, and two different inhibition zones can be evaluated [58].

As another modification of the disc volatilization test, Kloucek et al. [55] designed a multi-screening method performed in petri dishes divided into four sections to allow the simultaneous assessment of the susceptibility of up to four different microorganisms. Each section as well as the lid are filled with warm agar. After solidification, three parts of the dish are inoculated with different microorganisms; the fourth is left as a purity control. Then the solution of VA is placed on a round sterile filter paper disc, which is put onto the walls dividing the sections of the petri dish. For this purpose, a paper disc of a larger diameter (85 mm) was used in comparison with other methods. Finally, the petri dish is hermetically closed with the lid containing solidified medium. This relatively fast and simple screening assay method allows higher throughput than currently used methods performed in single petri dishes with different microorganisms seeded on one agar plate. A schematic design of the disc volatilization assay method and its modification is shown in ► Fig. 2.

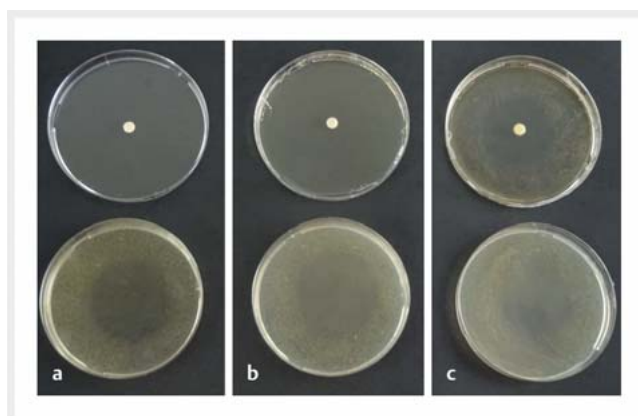
A disc volatilization assay can also be used in the evaluation of the combinatory activity of VAs. The interaction of VAs in the gaseous phase has previously been studied using this method by several researchers [59, 60], with the solidified medium in the petri dish exposed to the vapours of VA combinations placed on a paper disc. After incubation, zones of microorganism growth inhibition are measured on the agar surface. Subsequently, these zones are compared with the zones of individual compounds, or fractional inhibitory concentration indices (FICs) are calculated. However, this assay method based on a modification of the standard agar disc diffusion test is not appropriate for MICs determination [61] and suffers from a lack of automation [62].

Dressing model volatilization test

Edwards-Jones et al. [59] designed a more specified alteration of the disc volatilization method, modifying the matrix from which the compound tested is evaporated. This model can be used in the development of new wound healing preparations in medicine. The experiment is performed in a petri dish covered with various layers composed of different materials commonly used in the treatment of skin infections. Initially, the agar plate is inoculated with a bacterial suspension and covered with dressings, including a layer containing the VA (► Fig. 3). After an incubation period, inhibition zones are measured on the agar surface. This assay method simulates well the conditions of VA application in medicinal practice; however, its weakness is possibly the high level of interference of the tested agents with dressing models.

Airtight apparatus disc volatilization methods

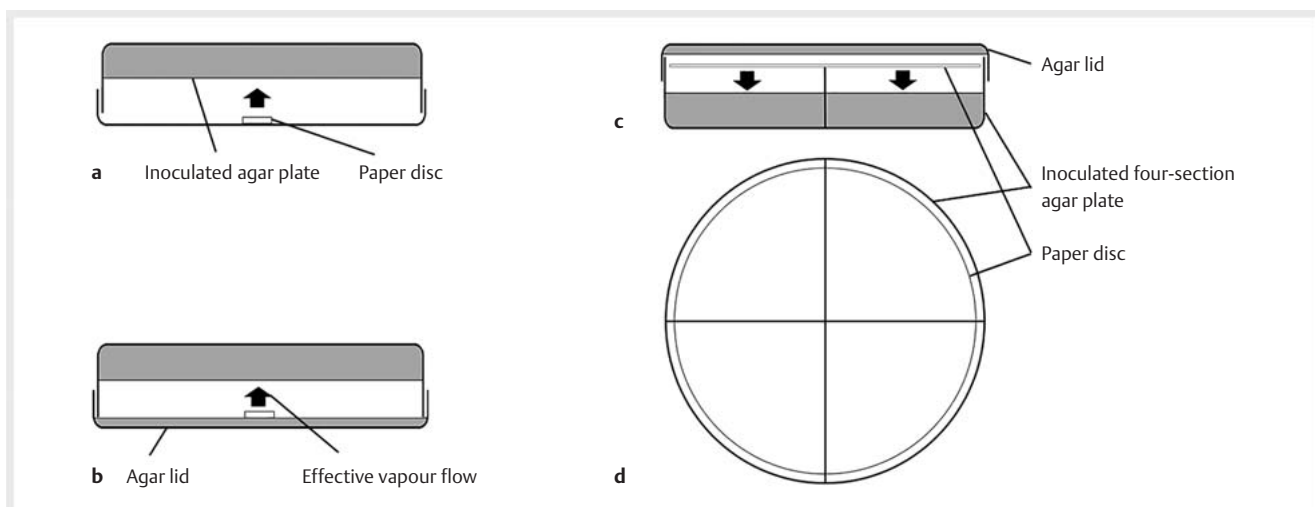
Inouye et al. [53] improved the disc volatilization assay method by using an airtight box, into which the petri dishes are placed (a method published under the name gaseous contact in an airtight box) as shown in ► Fig. 4. The inside part of the box is covered with aluminium foil to prevent plastic absorption of the VA and to protect the wall of the container from direct contamination by



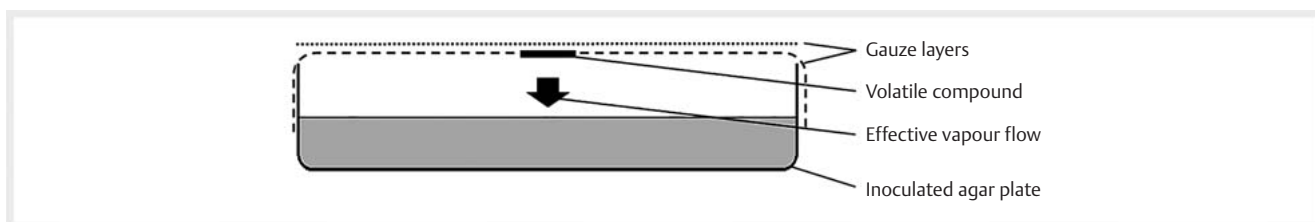
► Fig. 1 Disc volatilization method and its modifications, inhibition zones of carvacrol in a volume of 32 μ L (original author's photo). a Standard disc volatilization assay [57] with inoculated agar plate and paper disc containing the volatile compound on the lid. b Agar plate with agar sealing on the lid [54]. c Combination of disc diffusion and disc volatilization assay methods [58].

the VA. Paper discs are impregnated with VA solutions and inserted in the top of the airtight box apart from the petri dish with the inoculated medium. The authors of this method used 9 cm paper discs for the airtight box with a volume of 1.3 L. Another option is to insert the pure VA in a glass vessel inside the airtight box. Finally, the boxes are incubated under the required conditions. The advantage of this method lies in the possibility of using various inoculated materials and larger objects inserted into the airtight box to evaluate their surface decontamination. However, experiments with several boxes to evaluate the antimicrobial potential of EOs in different concentrations require a lot of space. Moreover, the location of the paper disc in the top of the airtight box and the distribution of vapours from top to bottom remain questionable.

To simultaneously assess the antimicrobial effect of volatile compounds at various concentrations, Seo et al. [63] constructed a special airtight experimental apparatus. It consists of an upper chamber with seven wells containing special nutrient agar medium (NGBA) with D-glucose and bromocresol purple as a pH indicator, which is inoculated with the bacteria to be tested, and a lower chamber with seven wells containing sterile paper discs with a twofold serial diluted liquid volatile compound. To avoid vapour leakage, O-rings are inserted at the juncture of the upper and lower well rims and around the whole set of wells; moreover, the four corners and centre of the apparatus are tightly sealed with nuts and bolts (► Fig. 5 a, b). After incubation, the growth inhibitory effect is evaluated by assessing the colour change in the nutrient agar from purple to yellow due to its response to any decrease in pH value caused by the growth of glucose-fermenting microorganisms, and the MIC is then determined. It is expected that this method could reduce the time required for the evaluation of the antimicrobial effect of VA in the gaseous phase, because it is possible to test several concentrations simultaneously. With the aim of facilitating sample preparation at specific concentrations, the volume of the headspace of the experimental appa-



► **Fig. 2** Schematic design of the disc volatilization method and its modifications. **a** Cross-sectional view of the standard disc volatilization assay [57] with inverted inoculated agar plate and paper disc containing the volatile compound on the lid. **b** Cross-sectional view of the inverted agar plate with agar sealing and paper disc containing the volatile compound on the lid [54]. **c** Cross-sectional view of the multi-screening disc volatilization method performed in a petri dish divided into four sections with a paper disc of larger diameter put onto the walls dividing the sections of the petri dish [55]. **d** Bottom view of the multi-screening disc volatilization method performed in a petri dish divided into four sections [55].



► **Fig. 3** Schematic diagram of the dressing model volatilization test [59] in a cross-sectional view.

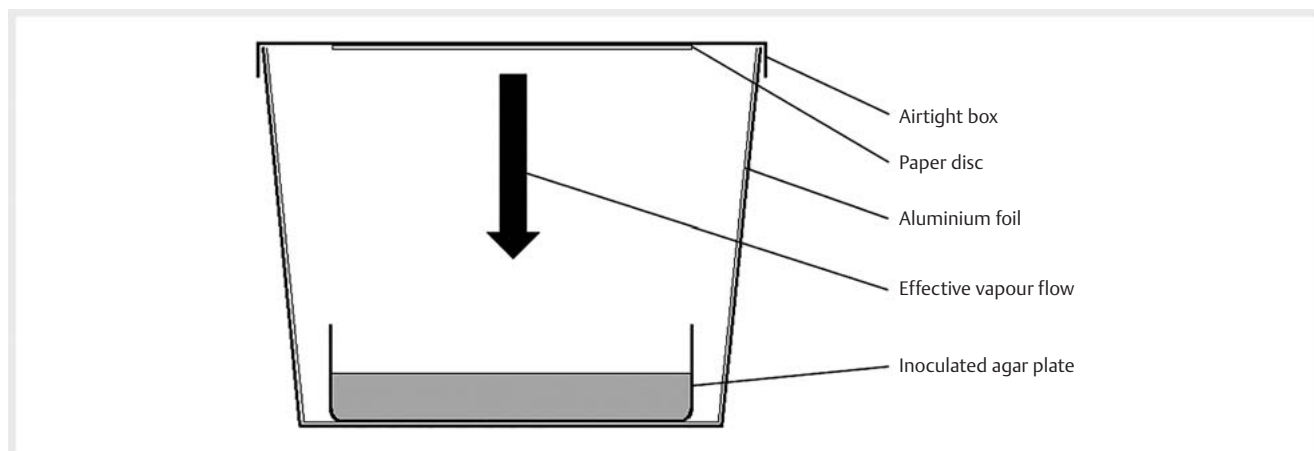
ratus was standardized to 1 mL. Nevertheless, this assay method requires special equipment that is not commonly available and NGBA visual inspection can only be applied to glucose-fermenting microorganisms.

Lee et al. [64] designed a modified version of above described experimental apparatus consisting of separated autoclavable polycarbonate vials. Similarly, the upper well contained a solid medium inoculated with bacterial suspension, and the lower well contained the VA gas generated from a sterile paper disc with serially diluted liquid compounds. Both parts were immediately put together and sealed with parafilm. To prevent leakage of VA gases, O-rings were positioned at the juncture of the upper and lower wells. A schematic diagram of an experimental vial is shown in ► **Fig. 5c**. Compared to the previous experimental apparatus [63] composed of seven wells, the vial format is easier to handle, as any number of samples in different concentrations can be simultaneously evaluated in the vapour phase using this experimental model.

Liquid matrix volatilization methods

Broth microdilution volatilization method

Recently, Houdkova et al. [65] designed a high-throughput screening assay method based on broth microdilution and disc volatilization methods. The experiments were performed on standard 96-well immune plates, covered by tight-fitting lids with flanges designed to reduce evaporation. Initially, agar is pipetted into every flange on the lid (► **Fig. 6a**) and inoculated with bacterial suspension after agar solidification. In the second part, seven twofold serially diluted concentrations of volatile compounds are prepared on a microtitre plate (this can also be performed using an automatic pipetting platform) and thereafter inoculated with bacterial suspensions. Finally, clamps, that are commonly available in do-it-yourself stores, are used for fastening the plate and lid together, with handmade wooden pads for better fixing (► **Fig. 6b**). The microtitre plates were incubated under specific conditions. The minimum MICs were evaluated by visual assessment of bacterial growth after colouring of the metabolically active bacterial colonies with thiazolyl blue tetrazolium bromide dye (MTT) when the interface of colour changes from yellow and purple is recorded in the broth and agar. The MIC values were deter-



► **Fig. 4** Schematic diagram of the cross-sectional view of the disc volatilization assay in the airtight box [53].

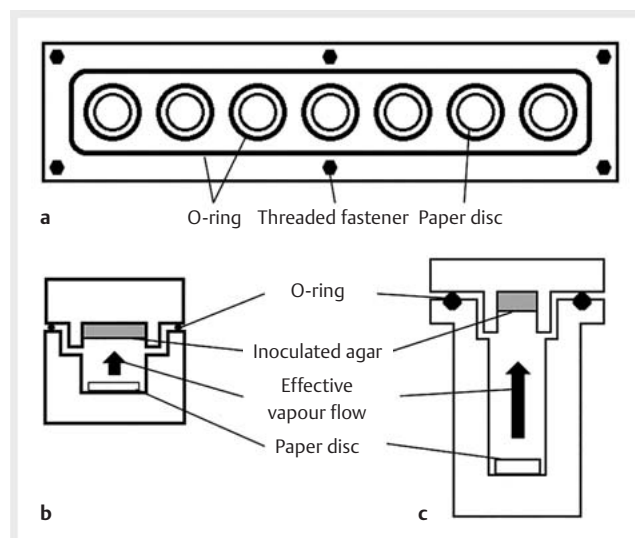
mined as the lowest concentrations inhibiting bacterial growth and expressed in $\mu\text{g}/\text{mL}$ (in the case of a vapour phase, also in $\mu\text{g}/\text{cm}^3$). A schematic design of the experiment is shown in ► **Fig. 7a, b**. A detailed cross-sectional view of a well of a microtitre plate with a flange on the lid is shown in ► **Fig. 7e**.

This assay method is suitable in the simple and rapid simultaneous determination of the antibacterial potential of volatiles in the liquid and vapour phases at different concentrations; several different samples may be assessed in one experiment. It allows for a cost and labour effective high-throughput screening of VAs without the need of a special apparatus. However, since the broth volatilization method described above is performed using serially produced microplates that are not designed for this purpose, the method suffers from several weaknesses. For example, clamps are necessary for fastening the plate and lid together and only a limited volume of agar can be applied on the lid, which could affect bacterial growth.

Netopilova et al. [66] modified the broth microdilution volatilization method for the evaluation of the combinatory effects of volatiles using a chequerboard design and allowing the determination of FIC indices. It differs from the method described above only in the layout of the assay plate (► **Fig. 7c, d**). In the case of testing combinatory effects, six twofold serial dilutions of one compound in horizontal rows are subsequently cross-diluted vertically by six twofold dilutions of the second compound using an automated pipetting platform. The combinatory effect of volatile compounds was determined based on the FIC.

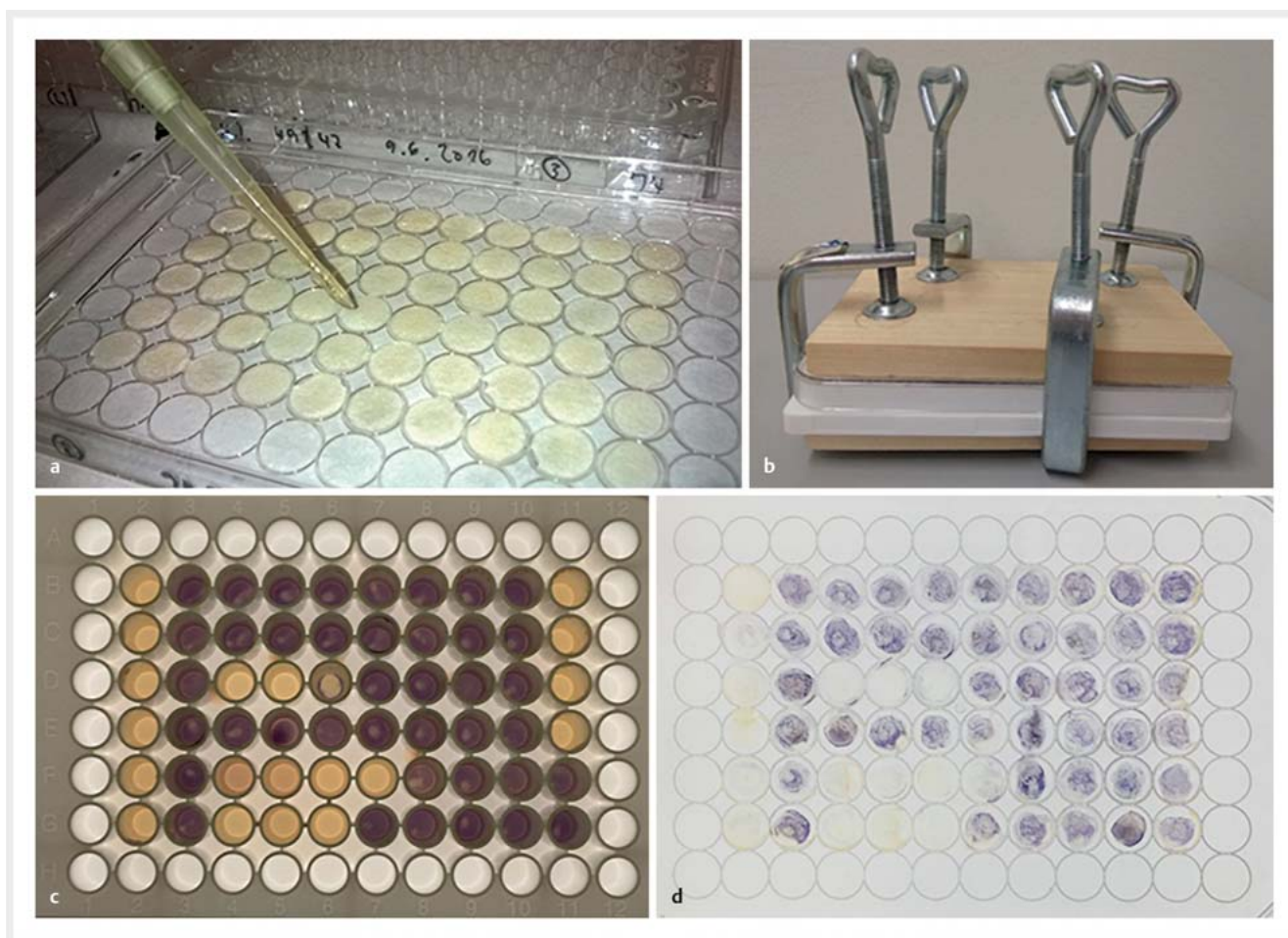
Microplate patch volatilization assay

Feyaerts et al. [67] introduced an assay method under the original name of vapour-phase-mediated patch assay for detecting vapour phase antimicrobial activity of the VA, which uses U-shaped, 96-wells microtitre plates, where a patch is defined as the set of wells in an area (square) surrounding one or more test wells. A schematic diagram of the plate design is shown in ► **Fig. 8**. First, microbial inoculum is added to all the wells and then the desired volume of the compound to be tested or its solution is added in the middle of a squared patch consisting of 9 or 36 wells. Wells lo-



► **Fig. 5** Disc volatilization assay method using a special airtight experimental apparatus [63, 64]. **a** A schematic diagram of the experimental apparatus (top view), **b** detail of one well of the experimental apparatus (cross-sectional view), and **c** detail of the special airtight experimental vial (cross-sectional view).

cated outside of the patch serve as internal negative controls. This well layout allows only one or two samples to be tested in one microtitre plate. Optionally, half of the patch and corresponding control wells can be sealed with a vapour barrier. Finally, the microtitre plate is covered with the lid and incubated in the required conditions. The results are evaluated by an optical density scan of each well as measured with a reader. This microtitre plate setup can be used to easily unmask false-positive results caused by the vapours; however, since it is not a quantitative method, it does not determine the exact values needed to assess the level of antimicrobial potential of the vapour phase.



► **Fig. 6** Microdilution volatilization method [65] (original author's photos). **a** Agar pipetting into every flange on the lid. **b** Use of clamps for fastening plate and lid together. **c** Colouring of the living bacterial colony with MTT in the plate (broth culture). **d** Colouring of the living bacterial colony with MTT on the lid (agar culture).

Agar plug-based vapour phase assay

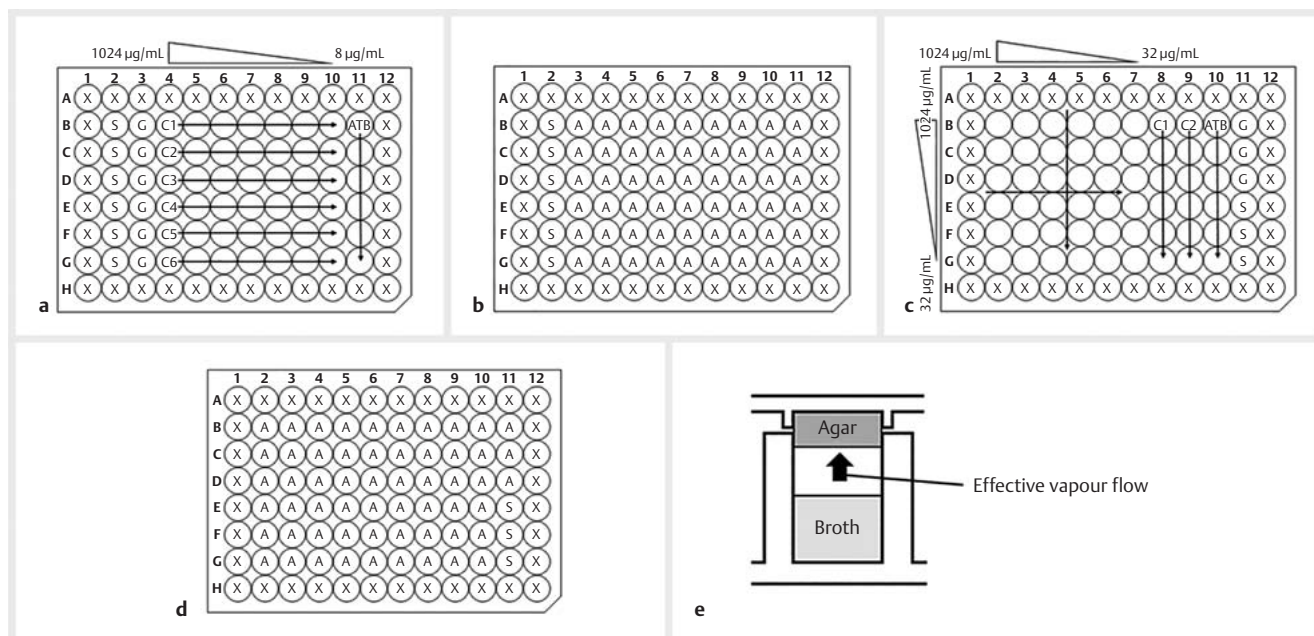
Amat et al. [50] developed an assay method providing both qualitative and quantitative measurements on the vapour phase antimicrobial activities of VAs. For this method, two separate agar plates are used. The first plate is inoculated with the pathogen and incubated for 1 h. Then agar plugs (13 mm in diameter) are obtained from this pathogen-seeded plate. A second plate has four parts of the agar removed (10 mm in diameter) where sterile caps from 1.5 mL disposable/conical freestanding microtubes containing the volatile compounds are inserted. The agar plugs prepared from the first plate are placed on the top of these caps (► **Fig. 9**). After 24 h incubation, the bacterial growth is examined visually. In the case of a quantitative evaluation of antibacterial activity, the agar plugs with bacterial cells are immersed into broth for 10 min and plated on agar to test cell viability and enumerate bacterial colonies compared with the growth control.

In comparison with the assays performed in petri dishes based on inhibition zone measuring, the agar plug-based vapour phase assay method, representing viable cell counting methods, provides more accurate data on reduced growth potential and it allows an evaluation whether the VA antimicrobial effect is biostatic

or biocidal. This model enables to test simultaneously several sample replicates against one bacterium or one volatile compound against different bacterial strains on one agar plate, while both options are applicable for a range of concentrations. However, preparation of the agar plugs may be labour and time consuming.

Airtight apparatus liquid volatilization method

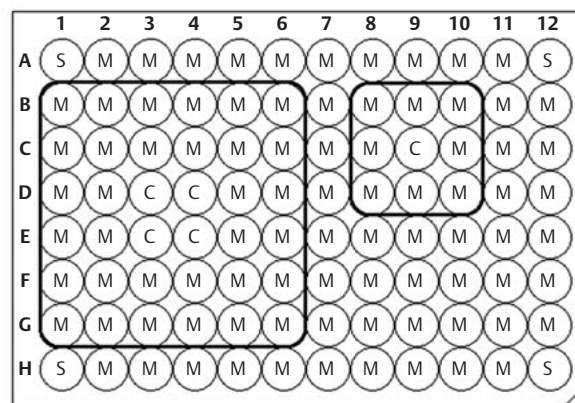
A method described by Sekiyama et al. [68], originally called the vapour-agar contact method, is performed in a sealed container containing an inoculated agar plate and a petri dish with the volatile compound to be tested (► **Fig. 10**). After incubation under the required conditions, inhibitory activity is evaluated by measuring the diameter of colonies formed by the pathogenic strains. Krumal et al. [69] improved on this experiment by inserting another petri dish in the container, which is filled with a solution of distilled water and sodium chloride to maintain constant relative humidity during the experiment. Similarly, like in case of the above described method using an airtight box, the distribution of the vapours and the final concentrations of the active compounds affecting bacterial growth on the surface of the agar plate is debatable.



► **Fig. 7** Schematic design of broth microdilution volatilization and broth volatilization chequerboard assay methods [65, 66]. **a, b** Microtitre plate and lid layout for testing six compounds. C1–6: seven serial twofold dilutions of tested volatile compounds; X: empty wells, not used in data calculation (problem of evaporation); S: sterility control (noninfected medium control; 0% growth of bacteria); G: growth control (100% growth of bacteria); ATB: serial twofold dilution of positive antibiotic control; A: agar. **c, d** Microtitre plate and lid layout for testing the combinatory effects of two compounds, where C1 is cross-diluted by C2. **e** Details of the cross-sectional view of one well of the microtitre plate with one flange on the lid.

Conclusions and Future Perspectives

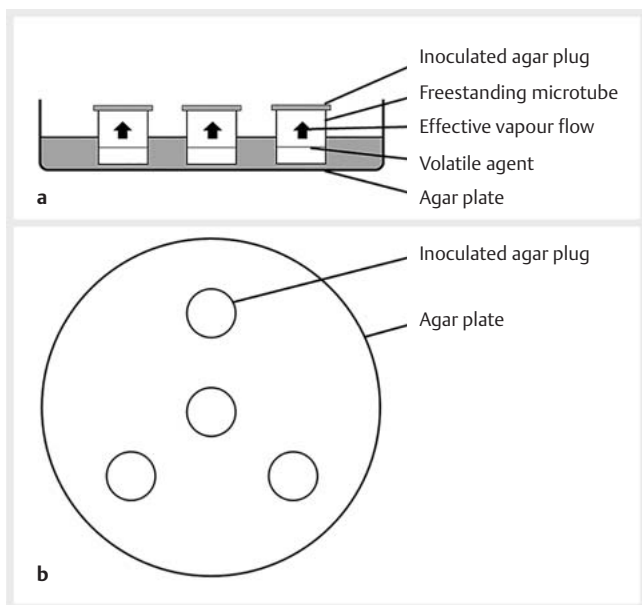
This review summarizes data on the *in vitro* antimicrobial effectiveness of VAs of plant origin (mainly terpenoids and EOs) and the *in vitro* methods used in the evaluation of their activity in the vapour phase. As a result of our literature analysis, the antimicrobial efficacy of vapours from 122 different plant species and 19 pure compounds examined in 61 studies using different *in vitro* tests against a broad spectrum of microorganisms was identified and summarized. According to the literature, 11 varied techniques and their modifications were developed to test the inhibitory effect of vapours on microbial growth. In this review, we proposed a classification of these methods based on the form of matrices to which the VAs to be tested are applied because the carrier medium/matrix selection is crucial for the volatilization of the tested agents. Seven of these assay methods work on the principle of the tested substances evaporating from a solid matrix (e.g., a paper disc). In case of the four other methods belonging to the second group, the samples are dissolved in solvents or they are tested in their pure liquid form. Of all the techniques found in this study, the disc volatilization assay method was the most commonly used in laboratory practice. Although this test is very simple to carry out, it has some disadvantages, such as a relatively high consumption of materials and labour, and its inappropriateness in determining the exact MICs. For the evaluation of the antimicrobial potential of VAs in the vapour phase, the broth microdilution volatilization assay method recently developed by Houdkova et al. [65], which is based on a standardized method recommended by the CLSI and EUCAST for the determination of the sus-



► **Fig. 8** Schematic diagram of the microplate patch volatilization assay [67], and plate layout of 9-well and 36-well patch; C: wells containing volatile compound solution; S: sterility control (non-infected medium control); M: microbial inoculum.

ceptibility of microorganisms to antimicrobial agents, may be more suitable.

The level of vapour transition from the matrix to which they are applied and their distribution into the inner atmosphere during the assay run are critical factors of volatilization assays that can significantly affect the results. For this reason, the concentrations in the vapour phase should be considered as indicative values on-



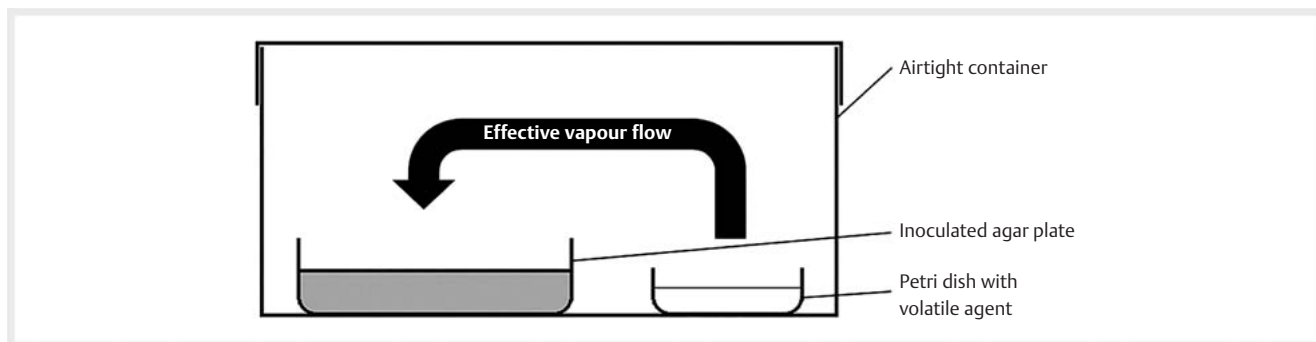
► **Fig. 9** Schematic diagram of the agar plug-based vapour phase assay [50]. **a** Cross-sectional view of the agar plate with microtubes containing the solution of the volatile compound and the inoculated agar plug on top. **b** Top view of the agar plate layout.

ly. In the case of uniform vapour distribution, the expression as a weight of VA per volume unit seems to be the most appropriate. For more accurate concentration determination, a headspace sampling technique that involves the use of a fibre coated with an extracting phase (known as a solid-phase microextraction) or a gas-tight syringe can be combined with gas chromatography analysis. Nevertheless, despite this approach, measuring especially the qualitative parameters, the quantitative assessment of the vapour phase remains problematic. The final interpretation of results obtained by *in vitro* antimicrobial assay methods should be critically evaluated, as has been previously reported by Kokoska et al. [29]. Only antibacterials with MIC values below 100 µg/mL for mixtures such as EOs and ≤ 16 µg/mL for pure volatile compounds should be considered as providing interesting activity,

whereas samples with respective MICs higher than 1000 µg/mL and 100 µg/mL should strictly be described as non-active. Testing samples in such large quantities should be excluded from the experiments. In addition to any reference antibiotic control, representatives of commercially used volatiles (e.g., thymol, carvacrol) should be involved in the assay design as a positive antimicrobial control.

In the future, *in vitro* techniques to evaluate the antimicrobial activity of VAs in the vapour phase can be useful for the development of novel antimicrobial preparations with practical application in various sectors such as medicine, pharmacy, the food industry, and agriculture, whereas their volatility will be their unique property that is advantageous over conventional antibiotics [70]. Their potential lies especially in inhalation therapies for the treatment of respiratory infections (e.g., pneumonia, tuberculosis, infections related to cystic fibrosis, and ventilator-associated infections), the preservation and shelf-life extension of food products using modified atmosphere packaging, and the protection of stored agricultural products as well as documents and exhibits in museums, archives, and libraries using a controlled atmosphere (fumigants). For example, the inhalation of the VAs could be an effective alternative treatment to some inhalation devices that are not appropriate for all patients (e.g., children, the elderly), as specific inhalation techniques and cognitive ability are required for the proper delivery of inhaled particles to the lung alveoli [71]. As can be seen in this review, several original apparatuses have previously been designed for antimicrobial susceptibility testing in the vapour phase. However, there is no specialized product based on these apparatuses currently on the market. Therefore, manufacturers of specialized laboratory consumables and equipment may also be interested in this topic.

Moreover, the development process of VA-based products should involve the determination of their safety to relevant normal human tissues, especially of respiratory and skin origin. An MTT colorimetric test previously described by Mosmann [72] is one of the most commonly used methods for *in vitro* evaluation of cytotoxicity using a microtitre plate design. This method is applicable to the toxicological assessment of natural products [73], including volatiles [74]. Volatile substances can influence the results of biological assays using a microtitre plate as has been de-



► **Fig. 10** Schematic diagram of the cross-sectional view of the airtight apparatus liquid volatilization method [68]. The inoculated agar plate and petri dish with the volatile compound solution are located on the bottom of the airtight container.

scribed by several authors [75, 76]. For this reason, it is necessary to modify the testing methodology and prevent the transmission of vapours, for example, by using an effective vapour barrier [65].

Previous research has been focused especially on the assessment of the antimicrobial potential of VAs obtained from relatively well-known medicinal, spice, and aromatic plants commonly used in traditional herbal medicine and as food condiments. Endemic and local plant species originating from tropical regions are considered valuable sources of antimicrobial agents. These plants have been less pharmacologically/phytochemically explored and they synthesize a wider spectrum of diverse compounds, including VAs, than the plant species of the temperate zones due to the stronger pressure of bacterial and fungal pathogens affecting plants in tropical ecosystems [29]. On the other hand, volatile substances such as EOs derived from plant species with a GRAS (generally recognized as safe) status based on their long history of use have great potential as natural preservatives in the food industry and agriculture, since the U. S. Food and Drug Administration (FDA) approved their safety for food application. Various studies evaluating the antimicrobial activity of some GRAS EO-bearing plant species or their constituents have been previously published [77–79], but only a few have directly focused on their vapour phase.

As far as methodological issues of research targeting the evaluation of the antimicrobial potential of VAs in the vapour phase are concerned, previous studies dealing with their efficacy have suffered from a lack of standardization, which is crucial for obtaining reproducible and comparable data. There are still no *in vitro* evaluations of the antimicrobial effectiveness of VAs that have been performed in accordance with the methods of the CLSI, NCCLS, and EUCAST. Since the techniques described in this review are limited by the specific weaknesses mentioned above, novel devices appropriate to overcome these disadvantages should be designed and brought to the market along with laboratory supplies. This represents a challenging task for research working in the area of VAs as well as a business opportunity for manufacturers of specialized scientific laboratory labware.

Acknowledgements

This research was financially supported by the Czech University of Life Sciences Prague (project IGA 20205001). The authors are grateful to Micheal Ua Seaghdha for providing language help.

Conflict of Interest

The authors declare that they have no conflict of interest.

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