Absinthe against multi-drug resistant bacterial pathogens? A recent update on the antibacterial effects of *Artemisia* compounds

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ABSTRACT

The widespread misuse of antibiotics leads to a rapid development of multi-drug resistant (MDR) bacterial pathogens all over the globe, resulting in serious difficulties when treating infectious diseases. Possible solutions are not limited to the development of novel synthetic antibiotics but extend to application of plant-derived products either alone or in combination with common antibiotics. The aim of this actual review was to survey the literature from the past 10 years regarding the antibacterial effects of distinct *Artemisia* species including *Artemisia absinthiae* constituting an integral component of the Absinthe drink. We further explored the synergistic antibacterial effects of the *Artemisia* plant products with established antibiotics. The survey portrays the *Artemisia* derived compounds as potent antibacterial agents that can even restore the efficacy of antibiotics against MDR bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *Escherichia coli*. This, in turn, is presumably triggered in part by the interaction of the *Artemisia* ingredients with the efflux pumps of MDR bacteria. In conclusion, biologically active molecules in *Artemisia* plants enhance the antibiotic susceptibility of resistant bacteria, which provide promising future therapeutic strategies to combat MDR bacterial pathogens.

KEYWORDS

*Artemisia*, Absinthe, antibacterial effects, efflux pump, novel antibiotic therapies, multidrug-resistant (MDR) bacteria, plant-derived natural products, traditional medicine

INTRODUCTION

The topic of antimicrobial resistance has increasingly gained the world’s attention in the past years. The World Health Organization (WHO) organizes an annual event, the World Antimicrobial Awareness Week, as part of the ‘Antimicrobials: Handle with care’ campaign, aiming to raise public interest about this serious issue. Importantly, in 2021, the topic ‘Spread awareness, stop resistance’ shed light onto the burden of multi-drug resistant (MDR) pathogens on the health care system, which, for the most part, results from the misuse of antimicrobials such as antibiotics, antivirals, antifungals and antiparasitics in human and veterinary medicine [1, 2]. Consequently, the WHO implemented a global action plan on antimicrobial resistance to address these serious health issues, particularly antibiotic resistance of distinct bacterial pathogens against several antibiotics [3].

Antimicrobial resistance has existed for a long time. In fact, the earliest evidence of resistance against β-lactam, tetracycline and glycopeptide antibiotics can be scientifically traced back 30,000 years [4]. In 1909, the antibiotic era was heralded by the discovery of the arsenic compound Arsphenamin (Salvarsan®) by Paul Ehrlich. Then, following discovery and medical application of the sulphonamides and penicillins, resistant bacteria arose limiting
treatment of infectious diseases. Nevertheless, the development of bacterial resistances constituted an ancient evolutionary phenomenon arising long before the discovery of antibiotics by mankind [5]. While primary (i.e., intrinsic) resistance is innate and based on bacterial characteristics, such as the glycopeptide resistance of Gram-negative bacteria, secondary resistance is acquired or adaptive. Additionally, acquired resistance can develop via mutations, through horizontal or vertical gene transfer, whereas adaptive resistance is a reaction triggered by environmental factors such as stress, growth conditions, pH, ion concentrations, and sub-inhibitory concentrations of antibiotics, for instance [6]. Adaptive resistance is unstable and results from epigenetic modifications of bacterial DNA. This renders bacteria versatile and highly adaptive to environmental changes [7]. Moreover, the rapid increase in drug resistance is not only due to the agricultural application of antibiotics, but also to the widespread misuse of antibiotics in human as well as veterinary medicine which leads to higher survival rates of bacteria, and the emergence of MDR pathogenic strains [8, 9]. This, in turn, leads to high mortality rates in the population due the increased complexity of treating these infections. This highlights the necessity to develop alternative or combinational therapies that target infections caused by MDR bacterial pathogens [10, 11]. One such promising approach might be the return to traditional medicine benefiting from thousands of years of empirical experience given that the antimicrobial effects of distinct natural products have been known for long.

The Artemisia genus belongs to the family of Asteraceae and includes approximately 250–500 species, most of which can be found in the Mediterranean region and Asia [12]. Plants of the Artemisia genus provide a high therapeutic potential and have been applied for various medicinal purposes since ancient times, including inflammatory conditions due to gastrointestinal and pulmonary infections [13]. For instance, Artemisia annua (also called Qinghaosu, Sweet Sagewort, Sweet Annie, Sweet Wormwood, Annual Wormwood) has been used to treat malaria for at least 1,600 years [14]. Another example, Artemisia absinthium (also known as absinthium, absinthesagewort, absinth wormwood and common sagewort) constituting the main ingredient in the popular Absinthe drink has been successfully applied in ancient Greece and in traditional medicine of Western Europe [15]. It is estimated that nowadays 70–95% of the human population worldwide relies on the health beneficial actions exerted by plant derived natural products in primary health care [16]. Particularly in Traditional Chinese Medicine natural products derived from Artemisia plants are of great medicinal impact. Furthermore, in compliance to worldwide guidelines an “Artemisinin-based combination therapy” (with artemether plus lumefantrine and artesunate plus sulfadoxine-pyrimethamine or dihydroartemisinin plus pipraquin) represents the pivotal treatment of malaria for long [17, 18]. Notably, in a murine model artemisone and artemiside was shown to control both, acute and reactivated disease following infection by the apicomplexan parasite Toxoplasma gondii [19].

Importantly, the primary ingredient of respective combinations is a compound of artemisinin, which constitutes an active agent in most of the plants of the Artemisia genus [20]. On top of that, many studies addressed the antimicrobial, anticancer, antiinflammatory, antipyretic, antiparasitic and antioxidant properties of plants from the Artemisia genus [14, 21]. These effects can be traced back to the biologically active molecules within the Artemisia plants, which have been identified in diverse compounds including flavonoids, monoterpenoids, sesquiterpenoids (e.g. artemisinin), coumarins, and aliphatic and lipid compounds [15, 22–26].

The primary goal of our literature survey was to provide an actual overview of the effects of Artemisia derived plant products and its biologically active agents on bacterial including MDR strains. We also aimed to assess the capacity of these different plant products to enhance the antibiotic susceptibility of MDR bacteria when combined with conventional antibiotics.

**METHODS**

**General inclusion and exclusion criteria**

We included studies i.) addressing the antibacterial effects of different Artemisia species (see Search query); ii.) focusing on different biologically active substances derived from Artemisia plants; iii.) assessing antimicrobial effects directed against defined bacteria by applying distinct methods. Studies addressing antimalarial effects were excluded manually. Furthermore, all studies referring to antihelminthic, antifungal and anticancer effects of Artemisia species had to be excluded as well as the investigations on chemical engineering of Artemisia plants. In order to provide an actual overview of knowledge we summarized the most recent findings from the past 10 years. Therefore, studies from before 2011 were excluded.

**Search query**

This literature survey was performed by using the MEDLINE database PubMed from September 14th to September 22nd, 2021. Our aim was to outline the topic as precisely as possible without excluding relevant studies.

First, we searched the database for publications that include the keyword “artemisia”. Therefore, we searched all fields through the Medical Subject Headings MeSH to ensure all terms were included, and combined them with the Boolean operator “OR”. Search #1 was “artemisia”[MeSH Terms] OR “artemisia”[All Fields]. For search #2 the target was antibacterial agents applying “anti bacterial agents”[Pharmacological Action] OR “anti bacterial agents”[MeSH Terms] OR “anti bacterial”[All Fields] OR “antibacterial”[All Fields] OR “anti infective agents”[Pharmacological Action] OR “anti infective agents”[MeSH Terms] OR (“anti infective”[All Fields] OR “antimicrobial”[All Fields]) AND “resist”[All Fields] to target multidrug resistance. Then, for search #4, we combined this
search with "Drug Resistance, Microbial"[Mesh] OR "Drug Resistance, Multiple"[Mesh] using the Boolean operator "OR", to ensure all the publications that target drug resistance are included. Finally, to limit the spectrum of results, we combined search #1, #2 and #4, using the Boolean operator "AND". The final search yielded 76 results. 24 studies related to malaria were excluded manually, five of which addressed phytotherapy and combination therapies which were not in the focus of our literature survey. Eleven studies investigating the physical or chemical properties of Artemisia species and the measures to increase the yield of artemisinin in a plant were excluded, which was also the case for 10 studies on the role of Artemisia plants in anticancer, antihelmintic or antifungal treatments. Furthermore, five studies addressing the biosynthesis of nanoparticles created from medicinal plants, and another two epidemiological surveys were also excluded. Another two surveys targeting from medicinal plants, and another two epidemiological studies addressing the biosynthesis of nanoparticles created in compliance with Charité practice [27] and adherence to legal data protection.

RESULTS

Antibacterial effects of Artemisia absinthium

Plant extracts have been used in traditional medicine for long and are important for inhibiting bacterial growth and biofilm formation, as well as for bacterial cytotoxicity and quorum quenching [12, 28]. Khan et al. assessed the role of different medicinal plants from Pakistan including Artemisia absinthium extracts against the "ESKAPE pathogens", namely Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterococcus faecium, displaying pronounced resistance rates. Interestingly, ethanol extracts of A. absinthium exhibited a dose-dependent antibacterial activity exclusively against the tested Gram-positive bacteria such as S. aureus and E. faecium with an MIC_{50} value of 256 µg ml^{-1}, resulting in at least 50% bacterial growth inhibition as tested by the broth microdilution method. The authors further assessed inhibition of bacterial quorum sensing and quorum quenching by using four reporter strains of S. aureus arg subtypes. Whereas a modest inhibition of quorum sensing could be measured with the ethanol as well as the aqueous extract of A. absinthium, no effect on quorum quenching could be observed. Furthermore, neither A. absinthium extract affected bacterial biofilm formation [28].

Another study conducted by Fiamigos et al., who investigated A. absinthium and its antibacterial effects on E. coli, Enterococcus faecalis and Bacillus cereus, revealed that chloroform extracts of A. absinthium alone did not exert any antimicrobial effects. However, when combined with 30 µg ml^{-1} of berberine, the growth of the aforementioned bacteria could be successfully inhibited. Among the major compounds of A. absinthium analyzed in terms of antimicrobial activity in this study, 4',5'-O-dicaffeoylquinic acid (4',5'-ODCQA), a dicaffeoylquinic acid isomer, was the only molecule shown to exhibit antimicrobial activity against the Gram-positive bacteria with a minimal inhibitory concentration (MIC) of 64 µg ml^{-1}. Importantly, 4',5'-ODCQA was identified as a specified efflux pump inhibitor for the Major Facilitator Super Family multi-drug efflux pumps of Gram-positive bacteria including S. aureus and E. faecalis [29].

Antibacterial effects of Artemisia annua

Artemisinin, a metabolite of the Artemisia annua plant, is known to possess potent antimicrobial and immunomodulatory features [30]. Four studies addressed the antibacterial effects of extracts and biologically active compounds of Artemisia annua [30]. In a study conducted by Goswami et al. [31], the authors investigated the antibiotic susceptibility of Helicobacter pylori towards artemisinin and its derivatives applying both, agar disc diffusion and broth dilution assays. The results showed that the compounds exhibited MICs of 2–8 µg ml^{-1} and minimal bactericidal concentrations (MBCs) of 4–8 µg ml^{-1} against H. pylori. Interestingly, artemether, an artemisin derivative, could completely overcome H. pylori drug resistance in this study. These compounds were also able to induce major deformations in the morphology of the H. pylori bacteria. Moreover, the antibacterial spectrum of artemisin and its derivatives was not limited to H. pylori, but was also extended to S. aureus, Staphylococcus epidermidis, Streptococcus mutans, B. subtilis, E. coli, and Enterobacter aerogenes [31].

Rolta et al. examined the synergistic effect of distinct antibiotic compounds in combination with the methanol and petroleum extracts of Artemisia annua directed against E. coli and S. aureus isolates [31]. The extracts did not only exert significant antibacterial activities by themselves, but also significant synergistic effects upon combination with defined antibiotics. For instance, for the methanol ether extract of A. annua MICs of 62.5 µg ml^{-1} against E. coli and S. aureus could be assessed, while the MICs of the petroleum ether extract against respective strains were 125 µg ml^{-1}. Against E. coli the MIC of both, chloramphenicol and kanamycin were 7.81 µg ml^{-1}, whereas vancomycin and erythromycin exhibited MICs of 250 µg ml^{-1} and 500 µg ml^{-1}, respectively. Chloramphenicol displayed a MIC of 125 µg ml^{-1} against S. aureus, whereas vancomycin, tetracycline and kanamycin showed a MIC of 250 µg ml^{-1} and erythromycin a MIC of 500 µg ml^{-1}. Upon combination, however, the methanol ether extract decreased the MICs of vancomycin, erythromycin, chloramphenicol and kanamycin by 8-fold in E. coli which was also true for S. aureus, while the MIC of tetracycline was decreased by 8-fold against E. coli and by even 15-fold against S. aureus. On the other hand, the petroleum ether extract decreased the MICs of vancomycin, erythromycin and tetracycline by 8-fold and, interestingly, the
MIC of kanamycin was decreased by 32-fold against E. coli. As for the MICS of vancomycin and erythromycin, they were decreased by 4- and 8-fold against S. aureus, respectively, whereas the MICS of chloramphenicol, tetracycline and kanamycin against S. aureus were decreased by 128-, 16- and 8-fold, respectively. This study also revealed that the main components in both extracts were phenolics, flavonoids, phytosteroids, alkaloids, glycosides, proteins and free amino acids [31].

In a very recent study, Golbarg and colleagues investigated the antibacterial properties of the essential oil, the aqueous and the ethanolic extracts of A. annua against MDR E. coli isolates by using microdilution and agar well diffusion assays [32]. The essential oil was the most potent plant product as it inhibited the bacterial growth with an inhibition zone diameter (IZD) of 20.0 ± 1.45 mm at a concentration of 11.11 mg ml⁻¹ (11.110 µg ml⁻¹), with a respective MIC of 10⁻⁴ mg ml⁻¹ (0.1 µg ml⁻¹), whereas the ethanol extract of A. annua exerted an identical MIC. When compared to distinct synthetic antibiotics, the essential oil was the most potent compound with an inhibitory effect of 56.7% when assessing all tested isolates and notably, exhibited antibacterial effects that were comparable to those observed with oxacillin, ampicillin, amoxicillin, amoxicillin-clavulanic acid, tetracycline, streptomycin, ceftriaxone, ciprofloxacin, cefuroxime, ceftazolin, cefazidime and cefixime. The authors further analyzed the phytochemicals in the extracts in more detail. The obtained results revealed the abundances of monoterpenes (alpha-pinene, camphene, 1,8-cineole, terpinene, Z-beta, cis-sabinene hydrate, borneol, Myrtenol, trans-(+)-carveol, and verbene), sesquiterpenes (alpha-copaene, trans-caryophyllene, germacrene, beta-selinene, bicyclogermacrene, caryophyllene oxide and ledene), diterpene, cycloalkanes, cycloalkenes, alkyne and aldehyde in the essential oil. The authors suggested that the reason for the enhanced antimicrobial potency of the essential oil might be due to the abundance of various antibacterial molecules. In both plant extracts, polyphenolic compounds including catechins and chlorogenic acid could be found, whereas in the aqueous extract only chlorogenic acid was detectable. Furthermore, the aqueous extract displayed higher catechin concentrations [32].

**Antimicrobial effects of Artemisia species directed against distinct bacterial species.**

**Mycobacterium species.** In the past years the antimalarial compound artemisinin has gained significant attention as a promising tuberculostatic drug [33, 34]. Martin et al. examined the antimicrobial effects of Artemisia afra and A. annua against Mycobacterium tuberculosis, Mycobacterium abscessus and Mycobacterium smegmatis [33]. Therefore, A. annua and A. afra were resuspended in dichloromethane acquiring extracts with a yield of 0.82% and ≤0.0077% of the active agent artemisinin, respectively. The MICS of the plant extracts of A. annua and A. afra against the tested mycobacterial species were 39 µg ml⁻¹ and <0.37 µg ml⁻¹ respectively, whereas the MIC of pure artemisinin was 75 µg ml⁻¹. These results indicate that the observed antimycobacterial activities cannot be traced back to the presence of artemisinin as an active agent only, but also to the combination of artemisinin with additional compounds, since upon increasing artemisinin concentration by 2-fold (i.e., from 150 µg ml⁻¹ to 300 µg ml⁻¹), the bactericidal effect did not change, meanwhile an equivalent increase in A. annua concentration significantly enhanced the bactericidal activity against M. tuberculosis. Furthermore, A. afra exhibited bactericidal activities to a lesser extent, which might be explained by the lower artemisinin concentration in the plant. The antimycobacterial effect of A. afra was, however, still more pronounced when compared to pure artemisinin. Importantly, the bactericidal properties of the plant extract were similar when compared to the effects exerted by antimycobacterial drugs, such as rifampicin, isoniazid, ethambutol, streptomycin and ofloxacin. Of note, A. annua exhibited significant bacteriostatic activities on the intrinsically more resistant M. abscessus strain, which was not the case for pure artemisinin and A. afra [33].

Furthermore, a study conducted by Gemenchu et al. addressed the antibacterial activities of methanol extracts of Artemisia abyssinica leaves against different M. tuberculosis and Mycobacterium bovis strains [34]. In this study, the extract exhibited MICS ranging from 6.25 to 50.0 µg ml⁻¹ against the M. tuberculosis and from 12.5 to 50.0 µg ml⁻¹ against the M. bovis strains under investigation. The extract showed a prominent antimycobacterial activity, but yet was less biologically active than the common antimycobacterial drug rifampicin [34].

**Escherichia coli.** In section Antibacterial effects of Artemisia annua, we have already reported the antibacterial effect of some Artemisia species against E. coli. In addition, Smirnova et al. investigated the effect of the plant polyphenols quercetin, rutin, tannin, catechin, naringenin and hesperetin contained in the Artemisia species A. austriaca and A. pontica [35]. Of note, this was the only study within this survey that did not reveal antibacterial activity, but, on the contrary, showed an even enhanced bacterial growth-promoting effect. In fact, the polyphenols did not exert synergistic effects with the tested synthetic antibiotics and, interestingly, the MICS even increased from 0.03 µg ml⁻¹ to 0.125 µg ml⁻¹ and to 0.25 µg ml⁻¹ for ciprofloxacin with polyphenols from A. austriaca and A. pontica, respectively. As for kanamycin, the MIC increased from 16 µg ml⁻¹ to 64 µg ml⁻¹ and to 128 µg ml⁻¹, which corresponded to a 4- and 8-fold increase for A. austriaca and A. pontica, respectively. This rather “protective” effect against the antibacterial activity of ciprofloxacin may have been due to iron metabolism recovery in the presence of the plant extracts. Additionally, the authors hypothesized that the antioxidant properties of the plant extracts contributed to this enhanced antibiotic resistance through several suggested mechanisms, including radical scavenging, iron chelation and inducing genes that encode antioxidant enzymes [35].

Further, a study conducted by Chebbac et al., focused on the antibacterial activity of the essential oil of Artemisia
negrei, a medicinal plant found in Morocco, against *E. coli* [36]. The essential oil displayed potent antibacterial effects against the two *E. coli* isolates tested, with MIC values of 6.25 μg ml⁻¹. Importantly, the essential oil of *A. negrei* contains a relatively high amount of oxygenated monoterpenes, which explains the high antioxidant and antimicrobial activity and suggests it as a potential source of radical scavenging [36].

**Antimicrobial effects of further Artemisia species**

As mentioned earlier, the *Artemisia* genus comprises a high number of species which can be found around the globe and exert significant antimicrobial, antioxidant and anti-inflammatory effects. In a study investigating the chemical composition of *Artemisia phaeolepis* and the antimicrobial activities of the essential oil and its major components [37], the obtained results revealed potent antimicrobial effects against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Listeria monocytogenes* and *Salmonella enterica*. The agar diffusion tests showed an IZD of >21.0 mm for the essential oil itself, whereas the active agents contained in *A. phaeolepis* eucalyptol and germacrene D resulted in an IZD of >20.9 mm and >22.2 mm, respectively. In fact, these tested compounds proved even more effective than the synthetic antibiotics streptomycin and tetracycline which induced IZDs of >20.0 mm and >21.5 mm, respectively. Caryophyllene oxide, another phytocompound, had the least IZD with <13.7 mm. When applying the broth dilution assays, the MICs of the phytocompounds camphor and terpine-4-ol were 31.25–62.5 μg ml⁻¹ and 62.5–125 μg ml⁻¹, respectively, whereas the essential oil MICs ranged from 62.5 to 125.0 μg ml⁻¹, which were comparable to the germacrene D MICs. Caryophyllene and caryophyllene oxide exhibited the least pronounced antibiotic activity with MICs >125 μg ml⁻¹. The results suggest that the pronounced antibacterial activities of most of the compounds under investigation and the essential oil of *A. phaeolepis* itself were due to the antibacterial potential of the monoterpenes. In the phytochemical analysis further phytocompounds of the *A. phaeolepis* essential oil were identified as sabine, β-pinene, limonene, linalool, borneol, spathulenol and thuione. The authors hypothesized that respective phytocompounds might additionally contribute to the antimicrobial effect of the essential oil and in fact, synergistic effects may not be ruled out [37].

Another study addressed the antibacterial effects of other *Artemisia* species against *P. aeruginosa* [38]. Therefore, the antibacterial capacity of the compound dihydroeucune (Dhl), a sesqueripene lactone contained in *Artemisia douglasiana*, was tested against distinct *P. aeruginosa* isolates. In fact, Dhl inhibited 50% of the bacterial growth of different *P. aeruginosa* strains, including the clinical MDR strain CDN118, at concentrations ranging from 120 to 480 μg ml⁻¹. The study also revealed Dhl MICs of 480 μg ml⁻¹ and 960 μg ml⁻¹ against *P. aeruginosa* reference strains, as well as a lower MIC of 280 μg ml⁻¹ when tested against the MDR strain, indicating that Dhl was bacteriostatic for virulent strains PA14 and DCN118, but bactericidal for the less virulent strains PA01 and PA103. Furthermore, Dhl did not only exhibit a significant antibacterial activity against the *P. aeruginosa* strains, but on note, also exerted significant synergistic effect upon combination with the synthetic antibiotics gentamicin, chloramphenicol and ciprofloxacin [38].

Another study examined the antibacterial properties of *Artemisia rupestris* and its components, namely the five flavonoids arteemetin, chrysoplenletin, pachypodol, penduletin and chrysoeriol highlighting the importance of developing resistance-modifying agents from natural products by investigating the synergistic effect of the compounds with existing antibiotics [39]. Upon combination with norfloxacin, chrysoplenletin, penduletin and chrysoeriol inhibited the bacterial growth of a fluoroquinolone-resistant *S. aureus* strain, reducing the MIC of the antibiotic by 4-, 16- and 4-fold, respectively. Interestingly, the combination of chrysoeriol and ciprofloxacin strongly inhibited the bacterial growth of one methicillin-resistant *S. aureus* (MRSA) strain, reducing the MICs by 128-fold from 64 μg ml⁻¹ to 0.5 μg ml⁻¹. Furthermore, chrysoeriol reduced the MIC of oxacillin against another MRSA strain by 8-fold from 128 μg ml⁻¹ to 16 μg ml⁻¹. Moreover, this study suggests that the molecular mechanism responsible for this synergistic antimicrobial activity, resulting from a drug efflux inhibitory effect, was accomplished by inhibiting the mRNA expression of the efflux pump or by directly binding to the NorA receptor [39].

Additionally, Choi et al. studied the antimicrobial effects of another *Artemisia* species, namely *Artemisia princeps*, against MRSA [40]. Interestingly, the ethanol extract of *A. princeps* showed a dose-dependent antibacterial activity, with significant inhibition at concentrations below 1 mg ml⁻¹ (1,000 μg ml⁻¹). Moreover, the extract strongly inhibited biofilm formation at concentrations >2 mg ml⁻¹ (> 2,000 μg ml⁻¹), with bactericidal effects observed at concentrations of 8–64 mg ml⁻¹ (8,000–64,000 μg ml⁻¹). The authors suggested that the molecular mechanism driving the antibiotic-resistance was due to the expression of distinct bacterial genes such as mecA-, sea-, agrA-, and sarA, which was significantly reduced by *A. princeps* at >1 mg ml⁻¹ (>1,000 μg ml⁻¹) [40].

Another study assessed synergistic antimicrobial effects of aqueous and methanol extracts from *Artemisia khorasanica* and the antibiotics amikacin and imipenem, as a potential alternative treatment option of infections caused by MDR *Acinetobacter baumannii* [41]. The authors showed that, by suppressing the efflux pump activity via competitive and non-competitive inhibition, the methanol extracts were more effective than the aqueous extracts, given that in combination with amikacin and imipenem, the methanol extract decreased the MICs against different *A. baumannii* strains by 4- and 8-fold, while the aqueous extract resulted in 4-fold decreases. Additionally, the observed synergistic effect exerted by the methanol extract was hypothesized to be mediated by inhibiting the efflux pump activity through competitive or non-competitive inhibition rather than diminishing the expression of distinct efflux genes such as *adeF* and *adeB* [41].
DISCUSSION

Summary

Since ancient times, secondary metabolites produced by medicinal plants have been widely applied to treat human diseases including infections [12, 42]. Additionally, the rapid emergence of MDR pathogens, partly due to improper use of antibiotics, is alarming and considered amongst the greatest challenges that the global healthcare systems have to face [12, 42]. As a result, finding new therapeutic approaches to combat the rise in drug resistance is urgent, particularly via agents that impede the antibiotic resistance mechanisms expressed by respective pathogens. Results of this literature survey demonstrate antibacterial activities for various compounds of several Artemisia species.

Some studies showed that the plant product exhibits a very low or even no antimicrobial activity when applied alone [28, 29, 31, 32, 39]. However, the products were synergistically active upon combination with established synthetic antibiotic drugs, even enhancing the efficacy of the tested antibiotics in some instances. These pronounced synergistic effects were observed in the combinations of artemisinin with metronidazole [30], petroleum and methanol ether extracts of A. annua with chloramphenicol [31], chrysosplenin, penduletin and chrysoeriol with norfloxacin, chrysoeriol with ciprofloxacin [39], extract of A. khorassanica with amikacin and imipenem [41] and the combination of DhL with chloramphenicol [38], for instance. Importantly, these results provide promising future options for the treatment of infectious diseases caused by MDR pathogens, whereby MDR strains of E. coli [32], A. baumannii [41], P. aeruginosa and K. pneumoniae [36, 38] and S. aureus [30, 39, 40] have become sensitive towards antibiotics when combined with the products derived from the Artemisia plants. In addition, some of the Artemisia compounds were equally potent as common antibiotics, as seen with artemisinin and artemisinic acid which are derived from A. annua, and were shown to exert antibiotic effects that were similar to those observed for clarithromycin against S. aureus and S. epidermidis [30], for instance. Moreover, the antibacterial effects of the plant extracts of A. annua and A. abyssinica on distinct Mycobacterium species, including M. tuberculosis and M. bovis, were virtually comparable to those observed upon application of synthetic antimycobacterial drugs [33, 34]. Some studies included in this survey revealed that the antibacterial effect of the plant product alone was even more pronounced when compared with established synthetic antibiotics. Among these highly potent plant products are the essential oils and to a smaller extent the ethanol extracts of the Artemisia plants. The antimicrobial potential of essential oils derived from A. annua and A. absinthium have been independently observed in two studies [43, 44]. Remarkably, the efficacy of the methanol ether extract of A. annua against E. coli and S. aureus [31] and that of the essential oil of A. annua against E. coli might be of pivotal clinical relevance, given that the antibacterial potency of the A. annua essential oil was comparable to the antibiotic effects exerted by 13 different synthetic antibiotics [32]. The pronounced antimicrobial effects of A. annua have been described before given its role in the treatment of malaria [45, 46]. Moreover, the essential oil of A. negrei was shown to be highly efficient when tested against MDR bacteria [36], which also held true for the ethanol extract of A. princeps [40] and the active agents eucalyptol and germacrene D, present in A. phaeolepis [37], and DhL found in A. douglasiana [38]. In fact, several studies indicated that the observed antibacterial effects can be traced back to the presence of monoterpenes and sesquiterpene lactones and possibly to a synergistic effect of the different phytocompounds present, which may explain the higher efficacy of essential oils compared to plant extracts or particularly active agents being tested. For that, we can assume that thujone, eucalyptol, germacrene D and DhL are particularly potent and promising for pharmacological use [36–38]. As a matter of fact, thujone constitutes a very important antimicrobial active agent, as it is present in many Artemisia species that exhibit antimicrobial activity (e.g. A. negrei, A. rupestris, A. phaeolepis, A. absinthium).

Some of the Artemisia species evaluated in our review have been examined in older publication. In a paper from 2002, for instance, Muyima et al. investigated the essential oil of A. afras as natural cosmetic preservative in aqueous cream formulations [47], whereas Setzer et al. addressed the antimicrobial activity of Artemisia douglasiana [48]. Furthermore, Nokerbek et al. reported potent antimicrobial effects of A. rupestris besides its cytotoxic and anticancer properties [49].

Conclusion and outlook

The here provided actual literature survey provides strong evidence that Artemisia plant products constitute promising antibacterial compounds that might be applied as “natural” alternatives to established synthetic antibiotics for the treatment of bacterial infections and as promising adjunct synergistic option to target MDR bacterial pathogens. Further investigations, particularly in vivo including clinical studies, are needed, however, to provide more robust evidence for antibacterial as well as immune-modulatory modes of action and prospective clinical application.

Limitations

Among the major limitations of this literature survey is the limited comparability of the studies included in this examination. The Artemisia species tested are numerous, although the species are similar in their phytocompounds; however, phytochemical analysis was not established in all of the investigations. In addition, the plant origins were different among studies, which in turn leads to a diverse phytochemical composition [50]. Moreover, the study designs including applied methods were different, whereby most of the studies investigated the methanol extract of the plant, but some also examined the aqueous extract, the ethanol extract, the hexane extract and the essential oil of the plant, for instance. Furthermore, another major limitation is that all included studies were in vitro investigations. Lastly,
this literature survey was performed by one investigator only and thus, mistakes cannot be excluded. Therefore, the results need to be evaluated carefully.

DECLARATIONS

Ethics statement: Not applicable (literature survey).

Conflict of interests: SB and MMH are Editorial Board members.

Authors’ contributions: JJ conceived and designed the survey, wrote the paper. NWS co-wrote the paper. SB provided critical advice in design of the survey, edited the paper. MMH supervised the survey, co-wrote the paper.

REFERENCES

31. Rolta R, Sharma A, Sourirajan A, Mallikarjunan PK, Dev K. Combination between antibacterial and antifungal antibiotics with


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