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Antibacterial effects of vanilla ingredients provide novel treatment options for infections with multidrug-resistant bacteria – A recent literature review

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REVIEW PAPER



ABSTRACT

Due to the increasing application of antibiotics not only in healthcare settings but also in conventional agriculture and farming, multidrug-resistant (MDR) bacterial pathogens are rising worldwide. Given the increasing prevalence of infections caused by MDR bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (ESKAPE pathogen complex), it is pivotal to explore novel alternative or adjunct treatment options such as phytochemicals with antibiotic properties. Vanillin and vanillic acid represent biologically active ingredients in vanilla that has been known for long for its health-beneficial including antimicrobial effects besides its role as flavoring agent. Therefore, we performed a literature search from the past 10 years summarizing the knowledge regarding the effects of vanilla constituents against bacterial including MDR pathogens. Our survey revealed that vanillin and vanillic acid exerted potent effects directed against distinct Gram-positive and Gram-negative bacteria by inhibiting growth, viability, biofilm formation, quorum sensing and virulence. Remarkably, when combining vanillin or vanillic acid with defined synthetic antibiotics pronounced synergistic effects directed against distinct pathogenic including ESCAPE strains could be observed. In conclusion, vanilla ingredients constitute promising alternative or adjunct options in the combat of infections caused by MDR bacterial pathogens.

KEYWORDS

vanillin, vanillic acid, phenolic compounds, phytochemicals, natural products, synergistic antimicrobial effects, antibiotic enhancers, novel antimicrobial therapies, multidrug-resistant (MDR) bacteria, ESCAPE pathogens

INTRODUCTION

Antibiotic resistance

Infectious diseases pose a major threat to public health. Bacterial infections have become treatable since the introduction of antibiotics at the beginning of the 20th century. The amount of antibiotics used has risen worldwide in the recent years. For instance, the consumption of antibiotic drugs increased by 35% from 2000 to 2010 with Brazil, Russia, India, China, and South Africa (BRICS) accounting for 76% of this increase [1]. In 2010 the usage of antibiotics in farming and veterinary medicine was conservatively estimated at 63,151 ($\pm 1,560$) tons with another estimated rise by 67% to 105,596 ($\pm 3,605$) tons until 2030, and a doubling in respective BRICS countries [2]. This growing (ab)use of antibiotics imposes the emergence of drug-resistant bacterial strains due to selection pressure [1]. In consequence,

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the prevalence of infections caused by multidrug-resistant (MDR), extensively drug-resistant and even pandrug-resistant bacterial pathogens is rising and this trend provokes the prescription of last-resort antibiotics, such as carbapenems and polymyxins including colistin [3]. Accumulation of carbapenem resistance is a particular problem especially among Gram-negative bacteria. For instance, results obtained from a study by Cai et al. revealed that in respiratory tract infections 50.3% of *Acinetobacter baumannii* and 19.4% of *Pseudomonas aeruginosa* isolates were resistant to carbapenems resulting in extended hospitalization and elevated mortality in the clinical setting [4]. *A. baumannii* and *P. aeruginosa* together with *E. faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter* species (spp.) form part of the so-called *ESKAPE* complex which represents the group of Gram-positive and Gram-negative bacteria that are responsible for increasing nosocomial infections and life-threatening morbidities [5].

Although MDR bacteria are nowadays acknowledged as major global threats to human health with progressive emergence, bacterial resistance to antibiotics is a rather old natural phenomenon that is mainly caused by the selection pressure of antimicrobials produced by microorganisms and plants during evolution. In their studies, D'Costa et al. reported abundance of resistance genes to β -lactams, tetracyclines and glycopeptide antibiotics in bacterial samples taken from 30,000-year-old Beringian permafrost sediments [6]. In fact, bacteria have been confronted with environmental threats for millennia and therefore, developed resistances due to Darwinian principles of evolution [6]. However, this problem *per se* is amplified by global antibiotic (ab)use in recent years. Overall, there are two major strategies of bacteria to adapt to the antibiotic "attack": First, by mutational events causing resistance and second, by horizontal gene transfer [7]. Mutational resistance emerges through mutations in genes that enhance cell survival in the environment, providing benefits to the cell versus susceptible bacteria. This can be achieved by (i) modifications of the antibacterial target resulting in a loss of affinity for the drug; (ii) a decrease in drug uptake by encapsulation or spore formation; (iii) an enhancement of efflux mechanisms to carry the harmful molecule out of the cell; or (iv) via the modulation of metabolic pathways [7].

Horizontal gene transfer is defined as the acquisition of foreign DNA material in different ways which is one of the most important drivers of bacterial evolution [7]. This can happen via (i) transformation, (ii) transduction, and (iii) conjugation. The latter represents an efficient way of gene transfer that involves cell-to-cell contact and is often observed in the emergence of hospital-related antibiotic resistance [7]. Recently, Kwon and Powderly postulated that the post-antibiotic era has already begun, given the progressive and highly dynamic rise in resistance development on one side and a virtual lack of novel antibiotic classes to fight infections caused by multidrug-, extensively drug- and even pandrug-resistant pathogens on the other side [8]. In this perspective a shortcoming of drugs available to prevent or treat bacterial infections leads to the view that

even routine surgical or chemotherapeutical interventions may be considered dangerous. Hence, it is pivotal to explore novel and innovative strategies to combat bacterial multi-drug resistance. In this context natural products including phytochemicals with antimicrobial and/or immune-modulatory properties will form part of alternatives or adjunct options for routine antibiotic treatment.

Biologically active ingredients in vanilla

Since centuries the vanilla bean *Vanilla planifolia* has been well-known for its healing properties in traditional medicine. Furthermore, its main constituents are used as preservatives and aromatics in the food industry. The plant contains more than 200 molecular components of which the phenolic aldehyde vanillin accounts for the main part of its medicinal activity with a share of one to two percent weight by weight (w/w) in cured vanilla pods [9]. Phenols are commonly abundant in nature and regarding their chemical structure, they consist of one or more benzene rings and hydroxyl group(s) [10]. Further, along with terpenoids, alkaloids, lectins and polypeptides, phenols constitute an important chemical class of phytochemicals long known for their antimicrobial effects which protect the plants against microbial infections [11]. Phytochemicals exhibit antibacterial effects, for example, by inhibiting biofilm formation or disrupting existing biofilms which are used by a large proportion of bacteria in nature to protect against antibiotics, and the resulting resistances pose an additional challenge in the treatment of human bacterial infections [12, 13]. One well-investigated antibacterial molecular mechanism of phytochemicals is the ability to interrupt bacterial quorum-sensing systems which are essential for regulating gene expression, virulence, and biofilm formation [11, 14]. In 2006, Choo et al. reported that the extract of *Vanilla planifolia* Andrews could effectively inhibit quorum-sensing in *Chromobacterium violaceum* [15].

Vanillin. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a phenolic aldehyde which was first isolated from vanilla in 1885 [10]. Nowadays, it is used as flavoring agent in food products, beverages and perfums [16]. It is estimated that in 2010, 15,000 tons of vanillin were sold all around the globe. It is interesting to note that only one percent of vanillin is extracted from vanilla pods [9]. A major part of vanillin is synthetically produced from guaiacol or lignin, and thus, it has become accessible for a wide range of use [10]. Vanillin consists of a phenol ring with an aldehyde-, a methoxy- and a hydroxy-group at specific positions. Besides its role as flavoring agent, vanillin has been proven to exert potent antimicrobial effects against distinct bacteria and yeasts [17] and furthermore, the molecule has antioxidant, anti-inflammatory, and even anticarcinogenic properties [18–20].

Vanillic acid. The hydrobenzoic derivative of vanillin, vanillic acid, is derived from vanillin by replacement of the aldehyde group with a carboxylic acid group. To date, anabolic, radical scavenging, and antioxidant effects of vanillic acid have been described, whereas the molecule could



exert pronounced antiinflammatory properties as shown for treatment of ulcerative colitis and cardiotoxicity in rats [21–23].

Objective

This literature review aims to summarize scientific reports on the antibacterial effects of vanillin and vanillic acid against MDR pathogens including bacteria of the *ESKAPE* complex. Furthermore, we intend to focus on potential synergistic effects of vanilla constituents if applied in combination with synthetic antibiotics.

METHODS

Inclusion and exclusion criteria

In this review we include the most recent (i.e., from the past 10 years) *in vitro* and *in vivo* studies addressing the antibacterial and antibiofilm effects of vanillin and vanillic acid. In addition, we included studies focusing on bacterial resistance, synergies with antibiotic compounds or investigations addressing *ESKAPE* pathogens. We excluded studies that examined (i) the antimicrobial properties of plant extracts, in which the individual effect of vanillin and vanillic acid as among other phytochemicals was not specified, and (ii) the effects of vanilla components on apathogenic bacteria in human and veterinary medicine, as well as farming or aquaculture.

Search

The literature search for this review was performed from June 28th, 2022 to July 20th, 2022 using the MEDLINE database PubMed. The exact process is shown in Table 1. For this review, a Boolean operator was used, which consisted of four major parts. First, the database was searched for the keyword “vanillin”. Thereby it became apparent that there was no MeSH term covering vanillin and its derivatives.

As a result we added the derivatives and synonyms “vanillic acid”, “vanillyl ester”, “vanillic acid”, “vanillate” and “vanillamides” and divided them with the operator “OR”. To ensure the keyword only be in the title or the abstract the tag “title/abstract” was added. The second part contained the keywords “antibacterial”, “antibiotic” and “antibiofilm” to assure that only studies focusing on the antibacterial effects were included. The third part was dedicated to bacterial resistances and the *ESKAPE* bacteria. Finally, the third and fourth searches were combined with the operator “OR” and finally added to *search #1* and *#2* by using the operator “AND”. All together 64 studies have been found.

After reading the abstract of these 64 studies, 26 were removed due to the exclusion criteria (see above). Of the remaining 38 studies, 13 were excluded because they addressed other topics, whereas three were excluded because they focused on other phenolic acids. Of the remaining 22 one was a retracted paper, and two publications were reviews. These were excluded as well. The remaining 19 studies were included and are commented in this review.

RESULTS

Antibacterial properties

In a recent study, Qian et al. [24] investigated potential antibacterial effects of vanillic acid on carbapenem-resistant *Enterobacter hormaechei*, which belongs to the Gram-negative *Enterobacter cloacae* complex. Using the agar diffusion method, the minimal inhibitory concentrations (MICs) were determined for four clinical carbapenem-resistant *E. hormaechei* isolates and the reference strain *E. hormaechei* ATCC 700323. The carbapenem-resistant *E. hormaechei* strain CREH-9 exhibited the highest MICs for meropenem (256 mg L⁻¹) and imipenem (512 mg L⁻¹) and was used for further testing. Vanillic acid exerted a MIC of 800 mg L⁻¹ against carbapenem-resistant *E. hormaechei* strain CREH-9 and hence, revealed significant

Table 1. Search query

Search	Query (using Boolean logic)	Results
#1	Search: "Vanillin"[Title/Abstract] OR "vanillyl ester"[Title/Abstract] OR "vanillic acid"[Title/Abstract] OR "vanillate"[Title/Abstract] OR "vanillamides"[Title/Abstract] Filters: in the last 10 years	2,918
#2	Search: "antibacterial"[Title/Abstract] OR "antibiotic"[Title/Abstract] OR "antibiofilm"[Title/Abstract] Filters: in the last 10 years	172,568
#3	Search: "bacterial resistance"[Title/Abstract] OR "mdr bacteria"[Title/Abstract] OR "multi drug resistance"[Title/Abstract] OR "antibiotic resistance"[Title/Abstract] OR "drug resistance"[Title/Abstract] OR "resistant bacteria"[Title/Abstract] OR "resistant"[Title/Abstract] OR "resistance"[Title/Abstract] OR "drug resistance, bacterial"[MeSH Terms] Filters: in the last 10 years	556,930
#4	Search: ("ESKAPE"[Title/Abstract] OR "enterococcus faecium"[Title/Abstract] OR "staphylococcus aureus"[Title/Abstract] OR "klebsiella pneumoniae"[Title/Abstract]) OR ("acinetobacter baumannii"[Title/Abstract] OR "pseudomonas aeruginosa"[Title/Abstract] OR "enterobacter"[Title/Abstract]) Filters: in the last 10 years	87,102
#5	Search: (#1) AND (#2) AND ((#3) OR (#4)) Filters: in the last 10 years	64

growth inhibition of the tested carbapenem-resistant *E. hormaechei* isolates at concentrations ranging from $0.5 \times \text{MIC}$ to $2 \times \text{MIC}$. Moreover, at vanillic acid concentrations according to the MIC or exceeding the MIC twofold, carbapenem-resistant *E. hormaechei* cells showed significant reductions in intracellular ATP and pH, indicative for a loss of bacterial cell membrane integrity. By using a field emission scanning electron microscope and transmission electron microscopy, Qian and colleagues further noticed significant anti-biofilm effects of vanillic acid at the MIC and the twofold increased MIC compared to the control group and furthermore, serious cell membrane damage to carbapenem-resistant *E. hormaechei* CREH-9 cells. Hence, vanillic acid exerts potent antibacterial effects directed against carbapenem-resistant *E. hormaechei* given bacterial cell membrane disruption and inhibition of biofilm formation along with decreases in carbapenem-resistant *E. hormaechei* cells within the biofilm [24].

Qian et al. [25] further tested the antibacterial activity of vanillic acid against carbapenem-resistant *Enterobacter cloacae*. However, this paper was retracted given two figures that had already been published in another publication focusing on the antibacterial effects of ursolic acid against carbapenem-resistant *E. cloacae* [26]. The authors asserted that their findings that vanillic acid exerted a significant antibacterial activity against carbapenem-resistant *E. cloacae* were nevertheless reliable [27]. In their study [25], the authors tested vanillic acid against the carbapenem-resistant *E. cloacae* isolate CREC-16, displaying a high level of antibiotic resistance due to production of both the *Klebsiella pneumoniae* carbapenemase (KPC) and the New Delhi metallo- β -lactamase-1 (NDM-1). The MIC of vanillic acid against CREC-16 was 600 mg L^{-1} and overall, vanillic acid showed similarly potent antibacterial effects on carbapenem-resistant *E. cloacae* when compared to carbapenem-resistant *E. hormaechei* in their previous study [24, 25]. Given decreases in intracellular ATP and pH levels indicative for cell death, vanillic acid also exerted pronounced antibacterial effects against carbapenem-resistant *E. cloacae* which also held true for biofilm formation and inactivation of bacterial cells within the biofilm [25].

Alvarado-Martinez et al. [28] addressed the antibacterial effects of vanillic acid, gallic acid, and protocatechuic acid against the Gram-negative enteropathogen *Salmonella enterica* serovar Typhimurium. All three compounds displayed pronounced concentration-dependent bactericidal effects with vanillic acid exerting a MIC of $1,500 \text{ mg L}^{-1}$ and a minimum bactericidal concentration (MBC) of 2000 mg L^{-1} resulting in a MBC/MIC ratio of 1.33. Moreover, incubation of *Salmonella* Typhimurium with sub-inhibitory concentrations of vanillic acid over 12 generations did not result in increases in the MIC which would have been indicative for resistance development. Concerning mechanisms of action, an increased uptake of propidium iodide by *Salmonella* Typhimurium cells after exposure to all three phenolic compounds pointed towards increases in membrane permeability. Additionally, the authors performed expression analyses of several *Salmonella* Typhimurium virulence genes.

While coinubation with gallic acid or protocatechuic acid resulted in downregulation of important virulence genes, vanillic acid itself did not exert such effects in a statistically significant manner. Conversely, vanillic acid even led to an upregulation of the *fliC* gene enhancing bacterial motility. The authors concluded that the impact of vanillic acid on *Salmonella* Typhimurium virulence remains still unclear, however a vanillic acid induced *fliC* upregulation might be indicative for cellular stress reactions in response to the disturbances in membrane integrity [28].

Another investigation evaluated antimicrobial properties of ten amides derived from vanillic acid [29]. Several amides were used together with (benzotriazol-1-yloxy)tripyrrolidionophosphonium hexafluorophosphate (PyBOP) as a coupling agent for derivatives 1 to 6 and dicyclohexylcarbodiimide (DCC) as a coupling agent for derivatives 7 to 10. When using the microdilution technique, all ten compounds showed antibacterial effects against *S. aureus* ATCC 25925 with MICs ranging from 3.14 to 4.48 mmol L^{-1} . No changes in MICs of respective compounds versus placebo could be determined when tested against *P. aeruginosa* strain ATCC 8027, *P. aeruginosa* isolate 102 and *S. aureus* isolate 47, however, indicative for lacking antibacterial effects [29].

Huang and colleagues [30] tested the antibacterial properties of Vanillic Acid-Loaded Core-Shell Nanospheres/Mesoporous Silica Nanoparticles (VA@Au-MSNs) as potential treatment option for orthopedic infections. These nanoparticles were produced by coating gold nanoparticles with mesoporous silica nanoparticles that were subsequently coated with vanillic acid. After coating the diameters of the nanoparticles increased from $101.1 \pm 8.5 \text{ nm}$ to $116.7 \pm 9.2 \text{ nm}$ indicating a loading efficiency of 18.56%. *In vitro* experiments revealed that the temperatures of the Au@MSN suspension increased up to 60°C after 12 s of irradiation with the near-infrared ray laser light (808 nm). The authors emphasized the importance of this effect given that $78.95 \pm 1.41\%$ of vanillic acid has been shown to be released at 42°C which is significantly higher than $42.62 \pm 1.71\%$ observed at 37°C . Furthermore, VA@Au-MSNs did not exert any cytotoxic effects when tested in different several cell lines. The authors then explored the antibacterial effects of VA@Au-MSNs against *S. aureus*. The obtained results revealed that coinubation with VA@Au-MSN and VA@Au-MSN irradiated with near-infrared ray decreased *S. aureus* cell numbers when compared to Au-MSN treated and control cultures. The authors concluded that the antibacterial effects against *S. aureus* due to vanillic acid released from the VA@Au-MSN might point towards a promising future therapeutic option in orthopedic infections [30].

Bernal-Mercado et al. [31] investigated the antibacterial and antioxidative effects of vanillic acid against uropathogenic *Escherichia coli* (UPEC). Vanillic acid alone exerted a MIC of 11.80 mM and a MBC of 17.84 mM against planktonic UPEC. Using the checkerboard method, a synergistic effect could be assessed upon combining 0.74 mM vanillic acid with 1.62 mM protocatechuic acid and 0.05 mM catechin. In addition, the antibiofilm properties of vanillic acid

were evaluated in UPEC and a minimum biofilm inhibitory concentration (MBIC) of 7.13 mM was determined. At this concentration cellular adhesion to silicon surfaces was shown to be entirely inhibited whereas planktonic cell growth was not affected. The combination of 0.74 mM vanillic acid, 1.62 mM protocatechuic acid, and 0.05 mM catechin again revealed a synergism resulting in a 6.89-, 9.83-, and 1398-times reduction of MBIC values for protocatechuic acid, vanillic acid, and catechin, respectively, if compared to singular application. With regard to biofilm formation of UPEC, a vanillic acid concentration of $2 \times$ MIC (25.95 mM) resulted in 100% inactivation of viable bacterial cells in biofilms. In addition, a combination of 2.97 mM vanillic acid, 3.2 mM protocatechuic acid, and 1.72 mM catechin led to an 100% inactivation of bacteria in pre-formed biofilms and further reduced the biofilm production of UPEC by 70%. In summary, vanillic acid when combined with protocatechuic acid and catechin exerts potent antibacterial effects against UPEC and constitutes a potent inhibitor of biofilm formation [31].

A study by Orlo et al. [32] assessed the antibacterial effects of vanillin, eugenol and capsaicin against *E. coli*, *P. aeruginosa*, and *S. aureus* isolates. Concentrations of vanillin inhibiting 50% of the bacteria were determined at 5.87 mM for *E. coli*, 9.12 mM for *P. aeruginosa* and 1.38 mM for *S. aureus*. MICs and MBCs were found to be 12.5 mM and 25 mM, respectively, against both *E. coli* and *S. aureus*. Against *P. aeruginosa*, the MIC of vanillin was 25 mM and the MBC 50 mM. The authors concluded that all three phytochemicals showed potent antibacterial properties against *E. coli*, *P. aeruginosa* and *S. aureus* isolates, whereas vanillin, however, was found to be less active compared to capsaicin and eugenol [32].

In a study conducted by Hussain et al. [33], vanillin derived 1,4-disubstituted 1,2,3-triazoles and *bis* 1,2,3-triazoles were synthesized and evaluated for their antibacterial activities. Therefore, vanillin was used as the main structural core and several derivatives of 1,2,3-triazoles (compounds 3a-f) or *bis* 1,2,3-triazoles (compounds 3g-k) were added. Next, the agar diffusion method was used to determine anti-bacterial properties against Gram-positive bacteria including *Bacillus subtilis*, methicillin-resistant *S. aureus* (MRSA) NCTC 10442 strain, *S. aureus* (ATCC25923), *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *E. faecalis*, vancomycin-resistant *Enterococcus* (VRE) species and Gram-negative bacteria such as *E. coli* (JM109), *K. pneumoniae* (ATCC33495), *P. aeruginosa* (ATTC15692) and *Shigella* species. Overall, the authors ranked the observed antibacterial effects of all derivatives as moderate to good. Compound 3g exerted the most prominent antibacterial effects given MICs of 5 mg L^{-1} against MRSA, *S. epidermidis* and VRE and 10 mg L^{-1} against *S. aureus*, *S. pyogenes* and *E. faecalis*. Ciprofloxacin was used as a control displaying MICs of 5 mg L^{-1} against *S. aureus*, *E. faecalis* and VRE and MICs of 10 mg L^{-1} against MRSA and *S. epidermidis*. Furthermore, compound 3g exerted weaker antimicrobial effects against Gram-negative bacteria given a MIC of 15 mg L^{-1} when tested against *K. pneumoniae*. The control compound colistin displayed MICs of 5 mg L^{-1} , 10 mg L^{-1} and 15 mg L^{-1} against *E. coli*, *P.*

aeruginosa and *K. pneumoniae*, respectively. The authors concluded that given the antibacterial effects against both Gram-positive and Gram-negative bacteria the derivatives of *bis* 1,2,3-triazoles may be promising candidates for new antimicrobial agents [33].

In their publication from 2018, Gao et al. [34] reported the design, synthesis, and antibacterial properties of vanillin hydroxamic acid derivatives. The compounds were synthesized using vanillin as a coupling agent between hydroxamic acid and indole derivatives and were thus classified as vanillin hydroxamic acids. In particular, compound 8 displayed strong antibacterial effects as indicated by a very low MIC of 0.32 mg L^{-1} against both *E. coli* (ATCC 10124) and *S. aureus* (ATCC 25923). Furthermore, a molecular docking study revealed that compound 8 shared the same binding interaction as actinonin, a known peptide deformylase inhibitor. Given a lack of toxic effects as revealed by *in silico* analyses the authors proposed the compound 8 as a promising antibacterial drug for future application in infection medicine [34].

The aim of a study by Vale et al. [35] was to evaluate potential synergistic antibacterial effects of ethylenediaminetetraacetic acid (EDTA) when combined the phytochemicals vanillic acid, quercetin, cuminaldehyde, and indole-3-carbinol against *E. coli* and *S. epidermidis* in planktonic and sessile states. First, the MICs were determined using the microdilution technique with vanillic acid presenting a MIC of 15 mM against both *E. coli* and *S. epidermidis*, whereas the MBCs were 25 mM for *E. coli* and 20 mM for *S. epidermidis*. The MIC of EDTA were $>25 \text{ mM}$ and 3 mM when tested against *E. coli* and *S. epidermidis*, respectively. However, no significant changes of the antibacterial activities could be determined when using EDTA in combination with vanillic acid against respective bacteria [35].

Synergies with common antibiotic compounds

A study focusing on antibiotic effects upon combinations of different drugs was performed by Brochado et al. [36]. Approximately 3,000 dose-resolved combinations of antibiotic substances, human-targeted drugs and food additives were tested against six strains of the three Gram-negative bacterial species *E. coli*, *P. aeruginosa*, and *Salmonella* Typhimurium. First, the results revealed that more than 70% of drug-drug interactions were bacterial species-specific, whereas 20% were strain-specific. In the case of vanillin and common antibiotics the authors discovered both, synergistic and antagonistic antimicrobial effects. For instance, increased protein levels of the efflux pump AcrA were detected in vanillin treated *E. coli*, resulting in elevated MICs of chloramphenicol and ciprofloxacin [36]. These results confirm data published by Abdelhamid and Abozahra [37], who found that an overexpression of the *acrA* and *mdfA* genes in *E. coli* resulted in increased resistance against levofloxacin. In contrast, Brochado et al. [36] observed a *mdfA*-dependent decrease of spectinomycin MIC against *E. coli* BW2511 upon addition of 100 mg L^{-1} vanillin and cells expressing *mdfA* were more susceptible to spectinomycin with vanillin in combination enhancing this effect.

Furthermore, a loss of synergy was observed in *E. coli* cells that did not express *mdfA*. In order to prove that the synergy of vanillin and spectinomycin was not related to vanillin's previously described effect on the efflux pump gene *acrA*, the same experiment was performed in single-gene knock-outs of the *acrAB-tolC* efflux pump gene. Finally, the synergism of vanillin and spectinomycin was further confirmed *in vivo* given an increasing survival rate of vanillin plus spectinomycin treated wax moths (*Galleria mellonella*) that had been infected by MDR *E. coli* [36].

Bezerra et al. [38] also addressed potential synergistic effects of vanillin in combination with common antibiotics directed against six strains of *S. aureus*, *E. coli*, and *P. aeruginosa* including MDR strains. Firstly, based on the fact that the MICs of vanillin against all six strains were $\geq 1,024$ mg L⁻¹, the authors concluded that vanillin was lacking a direct antibacterial effect. In case of *S. aureus*, however, a synergistic effect of vanillin in combination with gentamicin and imipenem could be observed, which in contrast, did not hold true for vanillin combined with norfloxacin, tetracycline or erythromycin against the same *S. aureus* strains. In case of *E. coli* the authors obtained similar results with vanillin decreasing the MICs of imipenem and gentamicin, with a lack of such combinatory effect in case of norfloxacin, tetracycline or erythromycin. Interestingly, decreased norfloxacin MICs could be assessed against *P. aeruginosa* upon combination with vanillin, while there was even an antagonistic effect when combining vanillin with tetracycline and erythromycin. Finally, combinations of vanillin with gentamicin and imipenem showed no enhanced antibiotic activity against *P. aeruginosa* [38].

In their study Oh and Jeon [39] investigated interactions of fluoroquinolones and macrolides with 21 phenolic compounds against the food-borne Gram-negative enteropathogen *Campylobacter jejuni*. Five phenolic acids (namely, vanillic acid, p-coumaric acid, sinapic acid, caffeic acid, gallic acid) and one flavonoid (taxifolin) exhibited synergistic antibacterial effects with both antibiotic substance classes. Adding 8 mg L⁻¹ vanillic acid to ciprofloxacin reduced the MIC by a factor of 4–16 when tested against five *C. jejuni* strains. Similarly, the MICs of erythromycin against *C. jejuni* decreased 2- to 16-fold following coinubation with 8 mg L⁻¹ vanillic acid. However, comparable results were obtained when adding the other phenolic acids [39].

Arya et al. [40] assessed the effects of vanillin capped gold nanoparticles (VAuNPs) in combination with common synthetic antibiotics against extensively drug-resistant *P. aeruginosa* strains PA11 and PA14. In order to increase its bioactivity of bioavailability, vanillin was added to VAuNPs as a reducing and capping agent. Chemically synthesized gold nanoparticules (CSAuNPs) were used as controls. First, the MICs of VAuNPs alone were determined to be higher than 2000 mg L⁻¹ and considered as non-bactericidal with reference to a paper by Simões et al. [11]. Next, the checkerboard assay containing several concentrations of antibiotics and VAuNPs was used to identify a concentration of 50 mg L⁻¹ VAuNPs and vanillin to be the basis for potentiation studies. When combining the antibiotics

chloramphenicol, levofloxacin, ciprofloxacin, tigecycline, meropenem, trimethoprim and fosfomycin with VAuNPs or vanillin, a reduction of respective MICs was observed in most cases. With regards to *P. aeruginosa* strain PA11, VAuNPs reduced the MICs of meropenem (10-fold) and trimethoprim (14-fold) when compared to the control. Similarly, for *P. aeruginosa* strain PA14, the MICs of trimethoprim could be lowered upon coinubation with vanillin by 3-fold and with VAuNPs by 4.8-fold. In contrast, no MIC reduction of meropenem was found when tested in combination with either vanilla constituent against the PA14 strain. In the case of *P. aeruginosa* strain PA14, VAuNPs were proven to be more effective than vanillin following combination with chloramphenicol, ciprofloxacin, trimethoprim, and tigecyclin. The same held true when tested against the PA11 strain given that the synergistic effects of VAuNPs in combination chloramphenicol, tigecycline, meropenem, and trimethoprim were more pronounced as compared to vanillin. Another aim of their study was to unravel the effects of VAuNPs and vanillin on the expression of the gene encoding for the MexAB-OprM efflux pump. The analyses revealed lower efflux pump gene expression levels in VAuNPs and vanillin as compared to CSAuNPs treated extensively drug-resistant *P. aeruginosa* strains PA11 and PA14 strains indicating that both compounds act as efflux pump inhibitors [40].

Anti-quorum sensing effects

By using global approaches Sivasankar et al. [41] performed transcriptomic and *in silico* analyses to find natural agents targeting the quorum sensing system of the Gram-negative enteropathogen *Yersinia enterocolitica*. A swarming assay revealed that vanillic acid at concentrations ≥ 200 mg L⁻¹ substantially attenuated bacterial motility, whereas migration was completely stopped by 400 mg L⁻¹ vanillic acid representing the minimal quorum sensing inhibition concentration (MQIC). When applying the broth microdilution assay, growth inhibition of *Y. enterocolitica* occurred with 500 mg L⁻¹ vanillic acid treatment. The authors further demonstrated that vanillic acid could inhibit 16% of cell-surface hydrophobicity constituting a factor for cell adhesion and pathogenesis. Moreover, production of extracellular polymeric substances (EPS) was reduced by up to 52% in vanillic acid treated cultures. EPS helps bacterial cells to develop durable biofilms and further supports adherence to host surfaces. To assess the viability of *Y. enterocolitica* towards human blood the study included a blood sensitivity assay. Results revealed that up to 92% of cells treated with vanillic acid died, whereas viability in non-treated cells decreased to 22% indicative for the potential of vanillic acid for treating bacteremia due to *Y. enterocolitica* infection. A transcriptomic analysis performed to evaluate the effects of vanillic acid on gene expression of *Y. enterocolitica* revealed that certain stress response genes such as superoxide dismutase genes, or the phage induced protein PspA, had been up-regulated significantly. In contrast, expression of distinct virulence genes modulating iron and

nitrite metabolism, efflux-transporters and motility was repressed. For instance, compared to the control the authors proved that flagella-related genes such as *fliJ*, *fliL*, *flgA*, *flgN*, *flgL* and *flgB* were not expressed in vanillic acid treated *Y. enterocolitica* and further, the expression of genes responsible for chemotaxis such as *cheB* and *cheR* were attenuated. Hence, a downregulation of such flagella- and chemotaxis-related genes may decrease motility and biofilm formation *in vivo*. Finally, Sivasankar et al. found that the gene encoding for the multidrug-efflux transporter yegM-NOB, also known as *mdtABCD*, to be downregulated in vanillic acid treated *Y. enterocolitica* which may lead to changing resistance patterns of common antibiotics. Therefore, the authors performed an antibiotic susceptibility testing by using the disc diffusion method and observed that following co-incubation with vanillic acid, the sensitivity of *Y. enterocolitica* towards gentamicin and amikacin changed from intermediate to susceptible, whereas sensitivities against ciprofloxacin, chloramphenicol, ampicillin and azithromycin were not affected by vanillin acid [41].

The aim of a study performed by Mok et al. [42] was to evaluate the effect of vanillin on quorum sensing in *P. aeruginosa*. A molecular docking study revealed that vanillin interacted with the active binding side of the *Pseudomonas* quinolone signal-binding response regulator PqsR constituting a regulatory unit in one of three interconnected quorum sensing systems in *P. aeruginosa*. The authors further showed that 1 mM vanillin could reduce the bacterial twitching motility, inhibit pyocyanin production and reduce the *Pseudomonas* quinolone signal (PQS) concentrations in planktonic cultures. Furthermore, adding 1 mM vanillin reduced the MIC of colistin on planktonic *P. aeruginosa* from 1 mg L⁻¹ to 0.5 mg L⁻¹. Moreover, combinatory therapy of vanillin and colistin enhanced the eradication of *P. aeruginosa* biofilms at a minimal biofilm eradication concentration of 1 mg L⁻¹ vanillin. The authors then confirmed these *in vitro* results *in vivo* by using the acute *Caenorhabditis elegans* infection model and found out that colistin in combination with vanillin increased the survival rate of *C. elegans*. The authors considered application of vanillin in the here applied concentration of 1 mM (0.015% weight by volume (w/v)) as safe given a use of far higher concentrations in food production and consumption (typically 2% w/v) [42].

The impact of plant phenolic compounds on growth and biofilm formation of *P. aeruginosa* was further addressed in a study by Plyuta et al. [12]. Hereby, the authors showed both, increased and decreased biofilm formation in vanillin treated *P. aeruginosa*, depending on the concentration. When growth of planktonic *P. aeruginosa* was reduced by 20–50%, biofilm formation was at its peak. The ability of *P. aeruginosa* PAO1 strain to form biofilm was enhanced by up to 7-times when vanillin was added in concentrations of up to 750 mg L⁻¹. However, both swarming and twitching motility of *P. aeruginosa* were not affected following co-incubation with vanillin, gallic acid or 4-hydroxybenzoid acid indicating that enhanced biofilm formation upon vanillin exposure was not related to increasing bacterial swarming or twitching motility. Interestingly, when adding 400–800 mg L⁻¹

of vanillin, the diameters of *P. aeruginosa* PAO1 swarming motility zones decreased by 50–60%. Even higher vanillin concentrations, however, resulted in inhibited biofilm formation, whereas also bacterial growth was restrained. One hypothesis for the enhanced biofilm formation by *P. aeruginosa* upon co-incubation of vanillin in subinhibitory concentrations could be that phenolic compounds mimic N-acyl-homoserine lactones (AHLs) as part of the *P. aeruginosa* quorum sensing system. To test this, the authors measured 3-oxo-C12-HSL and N-butanoyl-L-homoserine lactone (C4-HSL), two metabolites both of which synthesized by the two AHL-based quorum sensing systems. While synthesis of 3-oxo-C12-HSL was increased following application of vanillin, gallic acid and 4-hydroxybenzoic, the synthesis of C4-HSL was not increased. The authors concluded that phenolic molecules do not mimic AHLs and may modulate AHLs by interaction with the metabolism of *P. aeruginosa* PAO1 [12].

The antibacterial and antibiofilm effects of vanillin and syringic acid against methicillin-resistant *S. epidermidis* strains were further investigated by Minich et al. [43]. When using 1/20, 1/40 and 1/60 of the assessed MICs as subinhibitory concentrations, no effects on the cell viability in the biofilm were observed. The antibiofilm and inhibitory effects of both drugs on EPS production were further evaluated by crystal violet staining of three *S. epidermidis* strains, namely RP62a and two clinical isolates (745 and 817). Results revealed that vanillin applied at 1/20 of MIC reduced the biofilm formation of all three strains by more than 70%. Furthermore, vanillin significantly reduced the EPS levels in biofilms of all three *S. epidermidis* strains. Whereas the protein contents of the biofilms did not change upon vanillin treatment, increased sugar concentrations could be observed at all vanillin concentrations applied. The authors further asked whether vanillin would affect expression of distinct *aqr* genes involved in quorum sensing and EPS composition and found that in the presence of vanillin, the *agrA* and *agrD* genes, both of which under the control of the P2 promoter, were downregulated by 5-times if compared to the control group. The authors concluded that this attenuation of the quorum sensing signaling pathway by vanillin may result in the dampened biofilm formation as well as its changed EPS composition [43].

Further findings

An *in vitro* study conducted by Keman and Soyer [44] addressed the resistance development in *S. aureus* upon exposure to vanillic acid, 2-hydroxycinnamic acid (2-HCA) and vancomycin in subinhibitory concentrations. As part of the experiment the concentrations of vanillic acid, 2-HCA and vancomycin were increased after 24, 48 and 72 h of incubation. The initial MICs were 2.5 g L⁻¹ for vanillic acid, 1.6 g L⁻¹ for 2-hydroxycinnamic acid and 0.01 g L⁻¹ for vancomycin when measured against the applied MRSA and methicillin-susceptible *S. aureus* (MSSA) strains. Next, the bacteria were incubated with 1.3 g L⁻¹ vanillic acid or 1.2 g L⁻¹ 2-HCA. Vancomycin was used in concentrations of

0.005 g L⁻¹ for MRSA and 0.0075 g L⁻¹ for MSSA. The concentrations of vanillic acid and 2-HCA were increased by 0.1 g L⁻¹ per cycle, while 0.0005 g L⁻¹ vancomycin were added per cycle. The final concentrations used for incubation were 1.5 g L⁻¹ vanillic acid and 1.4 g L⁻¹ 2-HCA for both bacteria. Vancomycin was applied in concentrations of 0.006 g L⁻¹ for MRSA and 0.0085 g L⁻¹ for MSSA. Finally, MICs were determined after 48 h of incubation in an antimicrobial-free media. The final MICs of vanillic acid and 2-HCA remained unchanged when tested against both *S. aureus* isolates, whereas the MICs of vancomycin increased from 0.01 to 0.015 g L⁻¹ in case of MRSA and from 0.01 to 0.025 g L⁻¹ in case of MSSA. The authors concluded that resistance to both phenolic compounds could not be induced supporting the potential of vanillic acid and 2-HCA as a promising option for the treatment of infections caused by MDR pathogenic bacteria including MRSA [44].

DISCUSSION

Findings of the search

Overall, the results of this literature review revealed distinct antimicrobial effects of vanillin and vanillic acid on both, Gram-positive and Gram-negative bacteria, whereas in some instances, however, the findings were rather inconsistent. For example, vanillic acid exhibited direct antibacterial effects against Gram-negative bacteria such as *E. hormaechei*, *E. cloacae*, *Salmonella* Typhimurium and *E. coli* [24, 25, 28, 31, 35], which also held true for the Gram-positive bacteria *S. aureus* and *S. epidermidis* [30, 44, 35]. In case of vanillin, antibacterial effects against *E. coli*, *P. aeruginosa* and *S. aureus* were observed [32]. In contrast, two papers concluded that the obtained MICs were too high to consider antibacterial effects of vanillin or VAuNPs against *E. coli*, *P. aeruginosa*, and *S. aureus* [38, 40].

Interestingly, two studies applied vanillin as a structural compound for the development of future antibacterial drugs. Firstly, vanillin was combined with 1,2,3-triazoles or with bis 1,2,3-triazols resulting in antibacterial effects on Gram-positive and Gram-negative bacteria [33]. The second study used vanillin to synthesize derivatives of vanillin hydroxamic acid with effects against *S. aureus* and *E. coli* [34]. In view of the lower MICs measured, these new components may provide efficient antibacterial therapeutic options in the future.

Another important finding was the potential of vanillin and vanillic acid to interfere with the quorum sensing system of distinct Gram-negative bacteria, which resulted in a decrease in bacterial motility and biofilm formation [12, 41, 42]. One study detected an inhibition of quorum sensing and an antibiofilm effect in Gram-positive *S. epidermidis* by vanillin [43]. Other antibiofilm effects were upon coinoculation of carbapenem-resistant *E. hormaechei*, carbapenem-resistant *E. cloacae*, and UPEC with vanillic acid [24, 25, 31]. Furthermore, two studies addressed the potential resistance development in distinct bacteria following coinoculation with vanillin. However, no increases

in vanillin MICs could be observed when tested against *Salmonella* Typhimurium and *S. aureus* [28, 44].

In general, various synergistic, but also antagonistic effects could be assessed when combining vanilla ingredients with distinct antibiotic compounds. In *Yersinia enterocolitica*, for instance, vanillic acid exerted synergistic antibiotic effects when combined with ciprofloxacin, gentamicin, and amikacin, whereas in case of the latter two antibiotics, the sensitivity changed from intermediate to susceptible [41]. In the case of *E. coli*, lower MICs were measured for spectinomycin, gentamicin, and imipenem when adding vanillin [36, 38]. Increasing MICs, however, were identified when treating *E. coli* with vanillin and ciprofloxacin or chloramphenicol resulting in increased bacterial efflux pump expression [36]. A synergism of vanillin with gentamicin and imipenem was also demonstrated against *S. aureus* [38].

Furthermore, the MICs of norfloxacin and colistin decreased in combination with vanillin when tested against *P. aeruginosa* [38, 42], which also held true for meropenem, trimethoprim, chloramphenicol, ciprofloxacin, tigecycline, and levofloxacin when using gold nanoparticles covered with vanillin [40]. Conversely, however, antagonistic effects were observed in *P. aeruginosa* upon vanillin coinoculation with tetracycline or erythromycin [38]. Against *C. jejuni*, the activities of erythromycin and ciprofloxacin were increased by vanillic acid [39].

Finally, three studies evaluated the cytotoxicity of vanillin and VA@Au-MSNs in the concentrations used and considered the application of respective compounds as safe [30, 34, 42].

Conclusion and outlook

In conclusion, vanillin and vanillic acid display pronounced antibacterial effects by inhibition of growth, viability, virulence, and biofilm formation directed against distinct Gram-positive and Gram-negative pathogens including MDR strains. Further, there are potent synergistic effects when combining vanillin or vanillic acid with common synthetic antibiotic compounds. These results provide the basis for novel strategies to use phytochemicals including biologically active ingredients in vanilla as alternative or adjunct antibiotic option to treat infections caused by a significant number of bacterial pathogens including MDR strains. Nevertheless, more research both, *in vitro* and particularly *in vivo* needs to be undertaken to better understand the molecular mechanisms underlying the antibiotic effects exerted by vanillic acid and vanillin implemented in antibiotic drugs.

Limitations

The aim of this review was to survey the antibacterial effects of vanillin and its derivatives. It is important to underline the heterogeneity of the group of studies with different derivatives of vanilla constituents, applied methods and included bacterial strains. Given these heterogeneities, a direct comparison of the obtained results is rather difficult. Moreover, of the 19 publications reviewed, only two included *in vivo* experiments. Lastly, the search strategy was



done by a single investigator and therefore research mistakes cannot be excluded even though the search strategy was undertaken as carefully as possible.

DECLARATIONS

Ethics statement: Not applicable (literature survey).

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

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LIST OF ABBREVIATIONS:

AHL	N-acyl-homoserine lactone
BRICS	Brazil, Russia, India, China and South Africa
C4-HSL	N-butanoyl-L-homoserine lactone
DCC	dicyclohexylcarbodiimide
EDTA	ethylenediaminetetraacetic acid
ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> <i>Enterobacter</i> species
EPS	extracellular polymeric substances
2-HCA	2-hydroxycinnamic acid
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
MBC	minimum bactericidal concentration
MBIC	minimum biofilm inhibitory concentration
MDR	multidrug-resistant
MIC	minimal inhibitory concentration
MQIC	minimal quorum sensing inhibition concentration
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	methicillin-susceptible <i>Staphylococcus aureus</i>
NDM-1	New Delhi metallo- β -lactamase-1
PQS	<i>Pseudomonas</i> quinolone signal
PqsR	<i>Pseudomonas</i> quinolone signal-binding response regulator
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
spp.	species

UPEC	uropathogenic <i>Escherichia coli</i>
VAuNPs	vanillin capped gold nanoparticles
VA@Au-MSN	Vanillic Acid-Loaded Core-Shell Nanospheres/Mesoporous Silica Nanoparticles
VRE	vancomycin-resistant enterococci
w/v	weight by volume
w/w	weight by weight

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