FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food and Health 11(4), 330-341 (2025) • https://doi.org/10.3153/FH25028

*Research Article*

# Analysis of antibacterial and antibiofilm against foodborne pathogens and antioxidant activity of ethyl vanillin and characterisation of its ADMET profile

**Orçun TOKSÖZ**[1], **Denca TOKER**[2], **Didem BERBER**[3], **N. Cenk SESAL**[1]

**Cite this article as:**
Toksöz, O., Toker, D., Berber, D., Sesal, N.C. (2025). Analysis of antibacterial and antibofilm against foodborne pathogens and antioxidant activity of ethyl vanillin and characterisation of its ADMET profile. Food and Health, 11(4), 330-341. https://doi.org/10.3153/FH25028

[1] Marmara University, Faculty of Science, Department of Biology, Istanbul, Türkiye

[2] Marmara University, Institute of Pure and Applied Sciences, Department of Biology, Istanbul, Türkiye

[3] Maltepe University, Fine and Arts Faculty, Gastronomy and Culinary Department, Istanbul, Türkiye

**ORCID IDs of the authors:**
O.T.0000-0002-4863-3232
D.T. 0009-0003-1805-3063
D.B. 0000-0001-5813-160X
N.C.S. 0000-0002-0737-0122

**Submitted:** 19.03.2024
**Revision requested:** 15.05.2025
**Last revision received:** 20.05.2025
**Accepted:** 10.06.2025
**Published online:** 29.08.2025

**Correspondence:**
**Orçun TOKSÖZ**
**E-mail:** toksozorcun@gmail.com



## ABSTRACT

The number of outbreaks caused by foodborne pathogens is increasing annually due to poor hygiene practices in the food industry. Synthetic compounds are added to foods to extend shelf life and add flavour. Ethyl vanillin, a derivative of vanilla, is widely used as a flavouring agent. We aimed to determine the antibacterial and antibiofilm activities of ethyl vanillin on *Bacillus cereus* and *Escherichia coli* O157:H7, to determine its antioxidant potential (Folin-Ciocalteu and 1,1-diphenyl-2-picryl hydrazyl (DPPH)) and to analyse its chemical absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. The antibacterial activity of ethyl vanillin against *B. cereus* and *E. coli* O157:H7 was remarkably high, with inhibition ratios of 94.74 ±0.3% and 94.52 ±0.2%, respectively, at a concentration of 6000 µg/mL. The antibiofilm activity of ethyl vanillin against *B. cereus* was also high, with inhibition ratios of 92.03 ±0.04 and 92.9 ±0.65% even at very low concentrations. In addition, DPPH inhibition and TPC activity were found to be high. In this study, ethyl vanillin exhibited high levels of antibacterial, antibiofilm, and antioxidant activity. Ethyl vanillin was found to be acceptable for many properties according to ADMET analysis.

**Keywords:** Ethyl vanillin, Antibacterial, Antibiofilm, Foodborne pathogens, Antioxidant, ADMET

## Introduction

Concerns about food safety have increased in recent years with the rise of foodborne disease outbreaks. According to the report published by the World Health Organisation, 600 million people suffer from foodborne illnesses every year, unfortunately resulting in 420.000 deaths (WHO, 2022). Although the symptoms of foodborne illness vary depending on the type of microorganism, they usually include nausea, vomiting, abdominal cramps and, in particular, diarrhoea (Hassanain et al., 2013). The economic burden of contaminated food is known to exceed USD100 billion annually (Grudlewska-Buda et al., 2023).

Bacteria with biofilm-forming abilities pose a significant risk to the food and health industries. In this regard, the prevention and control of foodborne pathogens is of great importance for public health. The formation of biofilm by foodborne pathogens is a significant problem in numerous food processing industries, including dairy processing, food fermentation and fresh produce (Liu et al., 2023). The ability of pathogens to form biofilms renders them resistant to various factors, including ultraviolet (UV) radiation, high or low pH, salinity, and antibiotics (Yin et al., 2019). This resistance allows bacteria to maintain their biofilm structures, making them difficult to eradicate. As a result, the microbial load tends to increase, especially in food-related environments. This directly reduces the shelf life of food products and accelerates spoilage (Liu et al., 2023). When bacterial biofilms form in food, they become difficult to eradicate, resulting in serious economic and health problems that are often irreversible in the food industry. In addition, as bacteria become resistant to antibiotics, alternative methods are necessary to address cross-contamination (Olaimat et al., 2024). Biofilm-forming bacteria have been mostly encountered in the food industry, including *Enterococcus, Lactobacillus*, *Micrococcus*, *Streptococcus*, *Staphylococcus, Bacillus*, and *Pseudomonas* (Anand et al., 2014).

*Escherichia coli* O157:H7 and *Bacillus cereus* infections are recognised as among the most prevalent foodborne bacterial diseases transmitted to humans (Oluwarinde et al., 2023). These bacteria are also commonly identified as microorganisms associated with food contamination, especially in the dairy processing industry within the food sector (Ombarak et al., 2016). *B. cereus* is one of the bacterial pathogens that cause foodborne diseases in humans. This microorganism is a spore-forming bacterium which causes vomiting or diarrhoea. The health significance of this bacterium is also attributed to its ability to form biofilms. The spore and biofilm forms of *B. cereus* are of particular concern to the food industry as *B. cereus* can colonise and develop resistance on a wide range of surfaces and environments (Huang et al., 2020). Another important biofilm-forming and toxin (Shiga toxin) producing bacterium that threatens food safety in the food industry is *E. coli* O157:H7 (Öztürk et al., 2023). The biofilm formation of *B. cereus* and *E. coli* O157:H7 facilitates their persistence on food contact surfaces, leading to potential cross-contamination of food products (Nan et al., 2022).

Two primary methodologies can be employed to impede the formation of biofilms, which are known to elicit significant economic and health-related concerns. These methodologies can be categorised as physical eradication and chemical additives. Physical methods can be employed, including ultrasound, UV, pH, and temperature. Chemical methods are more economical and easier to implement than physical methods. These methods encompass the utilisation of various surfactants, disinfectants, herbal extracts and herbal metabolites. Of particular significance are herbal products, which are effective in preventing and eradicating biofilms without compromising food safety (Lu et al., 2022).

Vanilla, like saffron, is reported to be one of the most expensive spices (Morlock et al., 2021). It is obtained from the pods of *Vanilla planifolia* orchid, which has been reported to have approximately 200 compounds. The compound vanillin is responsible for flavour and fragrance (Arya et al., 2021). The sales value of vanillin is estimated to be USD 882.1 million in 2030. Furthermore, the global market value is predicted to reach USD 1,362.5 million by 2034, with a compound annual growth rate (CAGR) of 6.9% from 2024 to 2034 (Future Market Insights, 2024). However, vanilla cultivation has several disadvantages leading to economic losses due to *Fusarium* root and stem rot, anthracnose, and black rot (Kadir et al., 2019).

Vanilla extract is a commonly used flavouring ingredient in foods and beverages, consisting of a mixture of compounds other than vanillin. The production of vanillin is obtained from vanilla beans; however, the difficulty and high cost of obtaining vanilla are drawbacks for its widespread use. The price of vanilla beans, which are used to produce real vanilla extract, is relatively high, thereby increasing the cost. Therefore, synthetic vanillin and/or ethyl vanillin (3-ethoxy-4-hydroxybenzaldehyde) are dissolved in alcohol, propylene glycol, and/or glycerin to serve as artificial vanilla flavours (de Jager et al., 2007). Although ethyl vanillin is more expensive, it has a much stronger flavour than vanillin and is used as a flavouring agent (Fache et al., 2016). These compounds are also frequently used as food additives or in the composition of commercial foods such as ice cream, pastries, confectionery, candy, cookies, chocolate, and beverages to contribute to the odour/flavour of foods (Yemis et al., 2011). Vanillin has been reported in the literature to have antioxidant and antimicrobial effects (Banerjee & Chattopadhyay, 2019). Ethyl vanillin is also known to have antibacterial activity due to the presence of phenolic groups (Retnosari et al., 2021).

The phenolic aldehyde vanillin, ethyl vanillin and vanillic acid are generally regarded as safe and are used in foods such as pastries and confectionery (Yemis et al., 2011). The Chinese National Food Safety Standard legislation includes detailed information on the permitted limits for the use of vanillin and its derivatives. However, flavouring agents are not permitted in infant formulae intended for infants aged between 0 and 6 months. The maximum permitted levels of vanillin, ethyl vanillin, and vanilla extracts in infant and children's formulae with an age recommendation of over 6 months are 5 mg per 100 ml. Vanillin may be employed in complementary infant formula, with a maximum permitted level of 7 mg/100 g of the finished product (GB/T 2760-2014) (Wang et al., 2016). The JECFA (Joint FAO/WHO Expert Committee on Food Additives) reported the Acceptable Daily Intake (ADI) dose for ethyl vanillin as 0–3 mg/kg body weight (bw) (Peng et al., 2019).

This study aimed to investigate the antibacterial and antibiofilm properties of ethyl vanillin, a widely used compound in the food industry with known biological activities, against the foodborne pathogens *B. cereus* and *E. coli* O157:H7. At the same time, DPPH radical scavenging activity and total phenolic and flavonoid content of ethyl vanillin were determined, and computational analyses were performed by ADMET analysis.

## Materials and Methods

### Test Sample and Bacterial Strains

In this study, ethyl vanillin (CAS number 121-32-4) was investigated for its *in vitro* antibacterial and antibiofilm activities against foodborne pathogenic bacteria, as well as its in vitro total phenolic and flavonoid contents and antioxidant activities. Ethyl vanillin was dissolved in dimethyl sulfoxide (DMSO) to test its *in vitro* activity.

*B. cereus* and *E. coli* O157:H7, which were isolated from food samples, were obtained from Eurofins Scientific Food Analysis Laboratory.

### Antibacterial Test

The test bacteria were grown on Tryptic Soy Agar (TSA) at 37°C for 24 hours. Antibacterial activity tests of ethyl vanillin against the test bacteria were conducted in 96-well microplates using Tryptic Soy Broth (TSB). Overnight bacterial cultures were adjusted to an optical density (OD) of 0.02 at 600 nm in a 96-well plate. To test the antibacterial effect of ethyl vanillin, serial dilutions were made at concentrations of 6000, 3000, 1500, 750, 375, 187.5, 93.75 and 46.875 µg/mL. Tests were performed in triplicate. A Cytation 3 multimode microplate reader (Biotek) was used to measure the absorbance of bacterial growth after 24 hours for each bacterium (Mogana et al.,2020).

### Antibiofilm Activity

Antibiofilm activity of ethyl vanillin on *B. cereus* and *E. coli* O157:H7 bacteria was tested. Antibiofilm activity tests were performed by determining the concentrations at which no antibacterial activity was detected. According to the antibacterial activity results, concentrations in the range of 750-46.875 µg/mL for *E. coli* O157:H7 and 23.4-2.925 µg/mL for *B. cereus* were tested. TSB medium was prepared in 96-well microplates, and serial dilutions were made at appropriate concentrations of ethyl vanillin. The cultivated bacteria were incubated overnight at 37°C. The microplates were washed and kept at 60°C for drying. Then, 0.1% crystal violet was added to each well of the microplates for 10 min and washed three times before the second drying step. After drying, 33% acetic acid was added to each well. The OD values at 590 nm were recorded. The antibiofilm effect was calculated by comparing it with that of samples without ethyl vanillin added. Tests were carried out in three replicates (Karameşe & Dicle, 2022).

### Total Phenolic, Flavonoid Content and Antioxidant Activity

The spectrophotometric determination of total phenolic content (TPC) was done according to the Folin-Ciocalteu colourimetric method (Clarke et al., 2013). The TPC amounts of the aforementioned different tested concentrations of ethyl vanillin were determined. After mixing 100 µL of Folin-Ciocalteu reagent and 100 µL of ethyl vanillin in a 96-well microplate, each well was incubated with 7.5% $Na_2CO_3$ for 1 hour in the dark. At the end of the incubation period, the absorbance values for each sample were measured at 750 nm. The tests were performed in triplicate. Gallic acid solution was used to obtain a standard curve of total phenolic activity. The concentration of TPC was determined with the equation acquired from the standard curve of gallic acid.

Total flavonoid content (TFC) was determined according to the aluminium calorimetric method by calculating the rutin equivalence, and OD values were recorded at 435 nm (Yang et al., 2011). Absorbance values of ethyl vanillin concentrations were determined at five different concentrations after incubation for 15 min in 96-well plates with 1 M NaOAc. The TFC of the extracts was determined based on rutin equivalent per dry weight of the extract [mg Rutin equivalent (QE) g-1 dry extract weight].

The free radical scavenging activities of ethyl vanillin were determined using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay (Clarke et al., 2013). In a 96-well plate, 100 µL of DPPH solution and different concentrations of ethyl vanillin were mixed and incubated for 30 min in the dark. After incubation, the absorbance was measured using a Cytation-3 (Biotek) at 540 nm. L-ascorbic acid was used as a standard, and the experiments were performed in triplicate. The results were calculated using the equation below. DPPH scavenging effects of ethyl vanillin were expressed as a percentage.

DPPH% = [Acontrol-Asample) / Acontrol] x 100

***ADMET Analysis***

The two-dimensional structure and canonical SMILES of ethyl vanillin were obtained from the PubChem Compound page at https://pubchem.ncbi.nlm.nih.gov/. The usability potential of ethyl vanillin in food products was calculated with Admetlab 3.0 (https://admetmesh.scbdd.com/).

Physicochemical properties such as molecular weight, density, aqueous solubility (logS), lipophilicity (logP, logD), acid/base dissociation constants (pKa), melting point, and boiling point were determined to assess the stability and suitability of ethyl vanillin for formulation.

Medicinal chemistry values of QED (quantitative estimation of drug-likeness), GASA (general assessment of synthetic accessibility), NP Score (natural product-likeness), Lipinski's rule, colloidal aggregators, reactive compounds and promiscuous compounds were determined.

The absorption and distribution potential of ethyl vanillin was estimated through parameters such as PAMPA permeability, human intestinal absorption (HIA), oral bioavailability (F50%), plasma clearance (CLplasma) and biological half-life (T1/2).

Finally, toxicity and environmental safety profiles were assessed through TOX21 pathway predictions and toxicophore-based rules. Specific endpoints included hepatotoxicity (Human Liver Injury/DILI), nephrotoxicity (Drug-induced kidney injury), cytotoxicity (Hek293), bioconcentration factors, carcinogenicity, skin sensitisation, FDA maximum daily dose estimates (FDAMDD) and environmental parameters such as biodegradability and acute toxicity rules.

***Statistical Analysis***

Statistical analyses were performed in triplicate using One-Way ANOVA analysis of variance followed by Tukey's test. The significance of the treatments was determined at the $P \leq 0.05$ level. Different characters indicate statistical significance.

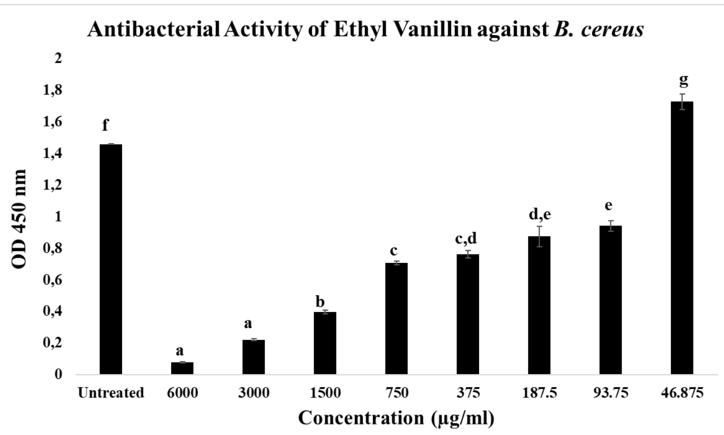
## Results and Discussion

Vanillin has been reported to include bioactive compounds with potential antioxidant and antimicrobial activity against bacteria and fungi in food products (Fitzgerald et al., 2004). In the literature, it is evident that there are more studies on vanillin than on ethyl vanillin. Ethyl vanillin can be used as a preservative in food formulations. It is a synthetic food additive soluble in ethanol, offering several advantages, including being more effective than vanillin for flavouring and serving as a substitute for natural vanilla. There are limited studies on the bioactivities of this chemical. Given that ethyl vanillin is used to replace vanilla in many food products, it is also important to evaluate its potential antibacterial, antioxidant and antibiofilm properties. The effectiveness in terms of antioxidant activity during the production stages of food products (storage and transport) has been reported (Tai et al., 2011a).

In this study, antibacterial and antibiofilm activities of ethyl vanillin against foodborne pathogens were investigated. In addition, total phenolic, flavonoid contents and antioxidant potential were evaluated using *in vitro* tests. Furthermore, the ADMET profile of ethyl vanillin was characterised to assess its safety and applicability in the food industry.

***Antibacterial Activity of Ethyl Vanillin Extracts against B. cereus and E. coli O157:H7***

The antibacterial activity of ethyl vanillin against the foodborne pathogen *B. cereus* was tested at eight different concentrations, ranging from 6.000 to 46.875 µg/mL. The results obtained demonstrated that ethyl vanillin exhibited significant antibacterial activity against *B. cereus*, with inhibition percentages of 94.74 ±0.3 at a concentration of 6000 µg/mL, 84.84 ±0.3 at a concentration of 3000 µg/mL, and 72.82 ±0.8 at a concentration of 1500 µg/mL. It was observed that antibacterial activity decreased at lower concentrations. The ethyl vanillin extracts exhibited moderate activity at a concentration of 750 µg/mL, with an inhibition percentage of 51.62 ±1.38. Conversely, lower concentrations, ranging from 375 to 93.75 µg/mL, demonstrated reduced activity in this experimental group, with inhibition values of 47.78 ±2.7%, 40.04 ±7.6%, and 35.5 ±4.04%, respectively. It was established that no antibacterial activity was present at the lowest concentration of 46.875 µg/mL (Figure 1).

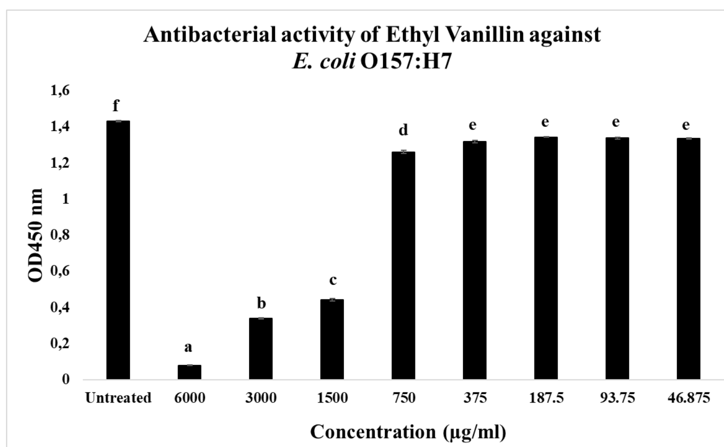



Statistical significance is indicated by different characters ($P \leq 0.05$)

**Figure 1.** Antibacterial activity of eight concentrations of ethyl vanillin extracts against the foodborne pathogen *B. cereus*

The inhibitory potential of ethyl vanillin extracts on the bacterial growth of *E. coli* O157:H7 was detected at the concentration of 6000 µg/mL, indicating the highest activity with an inhibition rate of 94.52 ±0.2%. In the 3000 µg/mL concentration, inhibition rates of 76.35 ±0.4% were observed, indicating high activity. Similarly, at the 1500 µg/mL concentration, inhibition rates of 69.17 ±0.9% were found to be highly active. Conversely, at lower concentrations ranging from 750 to 46.875 µg/mL, minimal antibacterial activity was observed against *E. coli* O157:H7 bacteria, with inhibition rates ranging from 11 to 6% (Figure 2).

In accordance with our data, similar results were also emphasised in the literature. Recently, Chen et al (2023) reported the inhibitory effect of vanillin on the bacterial growth of *E. coli* O157:H7. There are also studies in the literature that indicate the concentrations at which vanillin is effective. Ngarmsak et al. (2006) demonstrated that a minimum inhibitory concentration (MIC) of vanillin ranging from 10 to 13.3 mM was effective against several bacteria. They demonstrated that vanillin, at a concentration of 2 mg/mL, was effective in killing *E. coli* O157:H7. Arya et al. (2021) reported the antibacterial effect of vanillin against *Pseudomonas aeroginosa* at a concentration of 50 µg/mL. Cava-Roda et al. (2012) also demonstrated the antibacterial effect of 250–3000 ppm of vanillin on *E. coli* O157:H7 and *Listeria monocytogenes* in milk. Orhan-Yanıkan et al. (2019) recorded minimum bactericidal concentrations (MBC) at 2–6 mg/mL for vanillin against *Acinetobacter baumannii* and *E. coli*. Yemis et al. (2021) demonstrated potential inhibitory activity of vanillin, ethyl vanillin and vanillic acid on the growth of seven *Cronobacter* spp.. In our study, we found that ethyl vanillin exhibited significant antibacterial activity against *B. cereus* and *E. coli* O157:H7, with inhibition percentages of 94.74 ±0.3% and 94.52 ±0.2%, respectively, at a concentration of 6000 µg/mL. At concentrations of 3000 and 1500 µg/mL, the inhibition ratios were observed to be similar, 84.84 ±0.3% and 76.35 ±0.4% against *B. cereus*, and 72.82 ±0.8% and 69.17 ±0.9% against *E. coli* O157:H7, respectively. The findings of this study are consistent with the results reported in the literature. Furthermore, ethyl vanillin extracts exhibited moderate antibacterial activity at concentrations ranging from 750 to 93.75 µg/mL, with inhibition percentages ranging from 51.62 to 35.5%. These results demonstrate the effectiveness of ethyl vanillin even at concentrations below the ADI dose (0–3 mg/kg bw). The underlying mechanism may be due to the destructive effect of vanillin and ethyl vanillin on bacterial cell membranes, as Fitzgerald et al. (2004) have shown that these compounds cause a loss of the entire cytoplasmic membrane in both Gram-positive and Gram-negative bacteria. These findings were also supported by Scanning Electron Microscope (SEM) images in the study by Chen et al. (2023), which investigated the antibacterial effect of vanillin against *E. coli* O157:H7.

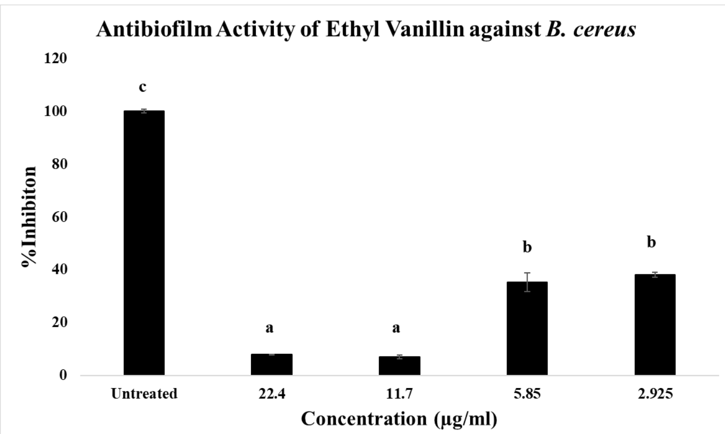



Statistical significance is indicated by different characters ($P \leq 0.05$)

**Figure 2.** Antibacterial activity of eight concentrations of ethyl vanillin extracts against the foodborne pathogen *E. coli* O157:H7

### *Antibiofilm Activity of Ethyl Vanillin against B. cereus and E. coli O157:H7*

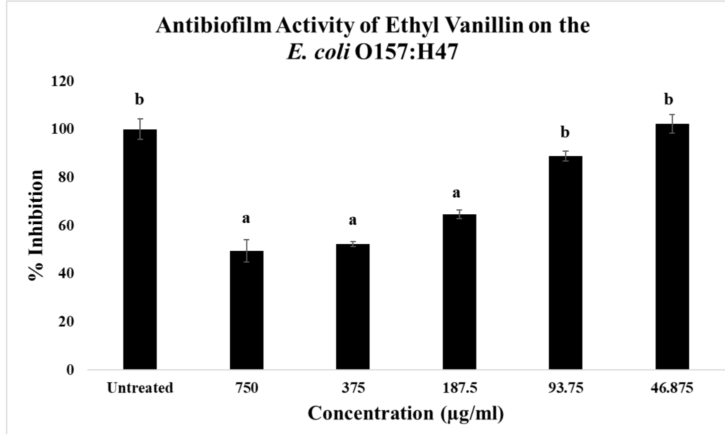
Antibiofilm activities of ethyl vanillin extracts against *B. cereus* were investigated for the concentration range of 22.4-2.925 µg/mL. These concentrations were selected because they did not exhibit antibacterial activity. Ethyl vanillin concentrations of 22.4 and 11.7 µg/mL were found to have a high level of antibiofilm activity of 92.03 ±0.04 and 92.9 ±0.65% against *B. cereus*, respectively. Ethyl vanillin extracts were found to have inhibitory activity on bacterial growth of *B. cereus* with the percentages of 64.71 ±3.56 and 61.9±1.04 at the concentrations of 5.85 and 2.925 µg/mL, respectively (Figure 3).




Statistical significance is indicated by different characters (P ≤ 0.05)

**Figure 3.** Antibiofilm activity of ethyl vanillin against the foodborne pathogen *B. cereus.*

The potential antibiofilm activity of the ethyl vanillin samples was also investigated against *E. coli* O157:H7. According to the results of the antibacterial activity tests, the antibiofilm activity of ethyl vanillin was tested at concentrations ranging from 750 to 46.875 µg/mL. The extracts of ethyl vanillin showed the highest antibiofilm activity against *E. coli* O157:H7 with the inhibition ratios of 50.56 ±4.57% at the concentration of 750 µg/mL and 47.71 ±1.06% at the concentration of 375 µg/mL. At lower concentrations of 187.5 and 93.75 µg/mL, biofilm activity was recorded as 35.36 ±1.76% and 11.09 ±2.08%, respectively. At the lowest concentration tested, 46.875 µg/mL, no activity on the biofilm formation of *E. coli* O157:H7 bacteria was found (Figure 4).




Statistical significance is indicated by different characters (P ≤ 0.05)

**Figure 4.** Antibiofilm activity of ethyl vanillin against the foodborne pathogen *E. coli* O157:H7

Since pathogenic bacteria adhere to food surfaces (stainless steel, glass, rubber, plastic, wood, etc.) and on food (vegetables, fruits, meat, seafood, etc.), causing the formation of biofilm, alternative sources for the prevention of biofilm formation can be considered as an alternative to conventional sanitisers (Orhan-Yanıkan et al., 2019). Thaweboon et al. (2017) tested ethanol extracts of vanillin at concentrations ranging from 0.5 to 20 mM against biofilm formation by *Candida albicans*. The researchers reported 75-80% inhibition of biofilm formation by *C. albicans* with vanillin extracts. Li et al. (2019) tested sub-MICs of vanillin on the biofilm formation of *Hafnia alvei,* and the biofilm inhibitory percentage of vanillin was found to be 68.5%. To the best of our knowledge, no study has been conducted on the antibiofilm activity of ethyl vanillin. In our study, ethyl vanillin was found to exhibit high antibiofilm activity against *B. cereus*, whereas this activity was at low levels against *E. coli* O157:H7. This may be due to differences in the cell membrane of Gram-positive and Gram-negative bacteria. It is well known that biofilm formation is also related to the quorum-sensing (QS) system and that N-acylhomoserine lactone (AHL) signalling molecules in the QS system play a role in microbial food spoilage and pathogenesis. Ponnusamy et al. (2009) found that vanillin inhibits AHL molecules linked to the QS system in *Aeromonas hydrophila*. The researchers showed moderate biofilm inhibition on polystyrene surfaces. Therefore, it is possible that ethyl vanillin may also have an effect on the QS system and may be effective against biofilm formation in food samples. Detailed studies on AHL-producing bacteria that cause spoilage in food samples can be conducted in this respect.

***Total Phenolic, Flavonoid Content and Antioxidant Activity***

DPPH radical scavenging activity and total phenolic content of ethyl vanillin in terms of DPPH inhibition (%) and TPC (mg GAE/g) at the concentrations ranging from 3000 to 46.875 µg/mL are presented in Table 1.

The DPPH inhibition percentage of L-ascorbic acid, used as the positive control, was found to be 88.05 ±0.1. The highest inhibition percentages of DPPH radical scavenging activity of ethyl vanillin were 90.72 ±0.3%, 89.03 ±0.73% and 85.9 ±1.45% at 3000, 1500 and 750 µg/mL concentrations, respectively. At the lower concentrations tested, ranging from 375 µg/mL to 46.875 µg/mL, moderate inhibitory activity was observed, with inhibition percentages of 77.92% to 55.16%.

In comparison with the other concentrations tested, TPC was found to be higher, with amounts ranging from 485.28 to 434.87 mg GAE/g in the concentration range of 1500-187.5 µg/mL. At concentrations of 3000 µg/mL and 93.75 µg/mL, the TPC of ethyl vanillin was moderate; however, at the lowest concentration tested, 46.875 µg/mL, it was 287.43 ±4.83 mg GAE/g. At concentrations between 3000 and 46.875 µg/mL, no flavonoid content was observed in ethyl vanillin.

**Table 1.** DPPH radical scavenging activity and Total Phenolic Content of Ethyl Vanillin

| Concentration (µg/mL) | DPPH Inhibition (%) | TPC (mg GAE/g) |
|---|---|---|
| 3000 | 90.72 ±0.3$^{d}$ | 334.04 ±19.33$^{a, b}$ |
| 1500 | 89.03 ±0.73$^{d}$ | 434.87 ±10.06$^{c}$ |
| 750 | 85.9 ±1.45$^{c, d}$ | 485.28 ±5.65$^{c}$ |
| 375 | 77.92 ±3.34$^{b, c, d}$ | 481.73 ±13.5$^{c}$ |
| 187.5 | 72.63 ±3.03$^{b, c}$ | 443.44 ±8.44$^{c}$ |
| 93.75 | 63.84 ±4.43$^{a, b}$ | 379.02 ±5$^{b}$ |
| 46.875 | 55.16 ±7.13$^{a}$ | 287.43 ±4.83$^{a}$ |

Statistical significance is indicated by different characters ($P \leq 0.05$).

Reactive oxygen species (ROS), which react with food components to form undesirable volatile compounds, destroy essential nutrients and alter the functionality of macromolecules, thereby adversely affecting nutritional, chemical and physical properties, are a concern in food storage and marketing (Choe & Min, 2006). Antioxidants have therefore traditionally been added to food formulations. Total antioxidant capacity is a crucial parameter for assessing bioactive components in foods. In our study, the total antioxidant activity (%) of ethyl vanillin was found to be considerably high at all tested concentrations, ranging from 3000 to 46.875 µg/mL. Furthermore, the total phenolic content of ethyl vanillin ranged from 334.04 to 485.28 mg GAE/g. From this perspective, the total antioxidant capacity of ethyl vanillin may be attributed to its phenolic compounds. There are limited studies on the antioxidant potential of ethyl vanillin. In a study conducted by Tai et al. (2011b), it was reported that ethyl vanillin and vanillin exhibited high antioxidant effects in the oxygen radical absorbance capacity (ORAC) assay. The study concluded that ethyl vanillin demonstrated significantly more vigorous antioxidant activity in comparison to vanillin. The researchers also reported that 20-25 µM of vanillin exhibited DPPH and ABTS inhibition, along with free radical scavenging. However, the results of studies on the antioxidant activity of vanillin are inconsistent in the literature.

***ADMET Analysis***

The physicochemical features, medicinal properties, absorption, excretion, toxicity, Tox21 pathway and toxicophore rules of ethyl vanillin were analysed by ADMET lab 3.0 (Table 2).

Ethyl vanillin had a molecular weight of 166.06 g/mol and a density of 0.968 MW/volume. The logarithm of aqueous solubility value (logS) was predicted to be -1.928, indicating that the solubility of ethyl vanillin in water was low. The logP (logarithm of the n-octanol/water distribution coefficient) and logD (logarithm of the n-octanol/water distribution coefficient at pH=7.4) values were found to be 1.514, indicating that it is more soluble in lipophilic structures. The pKa (acidic) value of ethyl vanillin was determined to be 8.533, and the pKa (fundamental) value to be 2.869. The boiling point was found to be relatively low, at 79.771 °C, while the melting point was found to be high, at 289.574°C (Table 2).

The medicinal chemistry-related parameters, namely QED, GASA, NPscore, Lipinski's rule, colloidal aggregators, reactive compounds, and promiscuous compounds, were also analysed. The QED (drug-likeness of desirability value) was found to be 0.694, and the GASA (synthetic accessibility score to estimate the ease of synthesis of drug-like molecules) value was found to be 0. The NPscore (natural product-likeness score) value, which shows the similarity of natural products, was found to be 0.337 (medium level). The Lipinski's rule value of 0.0 indicates an acceptable result. The Colloidal Aggregators value of 0.31 indicates that the solubility stability of this compound will be more reliable. The values for reactive compounds (0.994) and promiscuous compounds (0.556) were given in Table 2.

The absorption and excretion parameters of ethyl vanillin were evaluated using the Parallel Artificial Membrane Permeability Assay (PAMPA), HIA (human intestinal absorption of an oral drug) and F50% for absorption and CLplasma and $T_{1/2}$ for excretion (Table 3). The absorption characteristics of ethyl vanillin showed that it had low permeability with a PAMPA value of 0.26. The HIA value was 0.033, and the F50% value was 0.61, with an average bioavailability—the CLplasma (plasma clearance) value found to be moderate with a value of 9.038. The estimated half-life ($T_{1/2}$) was short at 1.546 hours (Table 2).

The toxicophore rules and TOX21 pathway parameters were also investigated. The Tox21 program predicts the potential adverse effects of chemicals via testing a 10,000-compound library (Ooka et al., 2024). Drug-induced liver injury (DILI) value was recorded as 0.334, which can be considered a moderate risk. The FDA maximum recommended daily dose (FDAMDD) is used to determine the amount of dose that should be taken daily. The value of FDAMDD was predicted to be 0.25 mmol/kg body weight/day. The values for skin sensitisation and carcinogenicity were 0.534 and 0.6, respectively. These values were considered to be intermediate toxicity. These values were regarded as average toxicity. The values for human hepatotoxicity, drug-induced nephrotoxicity and Hek293 cytotoxicity were 0.188, 0.073 and 0.269, respectively. These results indicate that the toxic effects are potentially low. The bioconcentration factor value was found to be 0.683, indicating that ethyl vanillin exhibited a low level of accumulation. SR-ARE, the antioxidant response element property, was also evaluated, and the value was found to be 0.003 (Table 2).

Additionally, the evaluation of the toxicophore rules for ethyl vanillin was investigated in terms of acute toxicity, skin sensitisation and non-biodegradability rule parameters. No potential acute toxicity effects were found for ethyl vanillin. The non-biodegradability rule was fulfilled. The skin sensitisation rule was predicted to be 5, indicating moderate potential for skin irritation (Table 2).

**Table 2** ADMET analysis of ethyl vanillin

| PHYSICOCHEMICAL PROPERTY | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Molecular Weight | Density | logS | logP | logD | pka (Acid) | Pka (Base) | Melting point | Boiling point |
| 166.06 | 0.968 | -1.928 | 1.514 | 1.514 | 8.533 | 2.869 | 79.771 | 289.574 |
| | MEDICINAL CHEMISTRY | | | | | | | |
| | QED | GASA | NPscore | Lipinski's Rule | Colloidal aggregators | Reactive compounds | Promiscuous compounds | |
| | 0.694 | 0 | 0.337 | 0 | 0.31 | 0.994 | 0.556 | |
| | | ABSORPTION AND EXCRETION | | | | | | |
| | | PAMPA | HIA | F50% | CLplasma | T1/2 | | |
| | | 0.26 | 0.033 | 0.61 | 9.038 | 1.546 | | |
| TOXICITY AND TOX21 PATHWAY | | | | | | | | |
| DILI | FDAMDD | Skin Sensitization | Carcinogenicity | Human Hepatotoxicity | Drug-Induced Nephrotoxicity | Hek293 Cytotoxicity | Bioconcentration Factors | SR-ARE |
| 0.334 | 0.25 | 0.534 | 0.6 | 0.188 | 0.073 | 0.269 | 0.683 | 0.003 |
| | | | TOXICOPHORE RULES | | | | | |
| | | | Acute Toxicity Rule | Skin Sensitisation Rule | NonBiodegradable Rule | | | |
| | | | 0 | 5 | 0 | | | |

In recent years, the application of ADMET, which determines the pharmacokinetic and pharmacodynamic properties of ligand molecules, has played a vital role in drug discovery and development. ADMET is utilised in numerous studies as an online server for toxicity prediction, drug similarity, and molecular descriptor calculation. These programs are mainly used in the pharmaceutical/medical industry. On the other hand, they can be helpful in the food industry for obtaining detailed information on the characterisation of compounds in terms of their composition, toxicity, and potential activities. In the present study, the physicochemical properties, medicinal chemistry, absorption, excretion, and toxicity of ethyl vanillin were analysed using ADMET Lab 3.0. The program predicted that the solubility of ethyl vanillin in water is low and that it is more soluble in lipophilic structures. The boiling point was found to be relatively low, at 79.77 °C. This suggests that it may be more protective in foods with relatively high lipid content. The fact that the boiling point is a little low is actually questionable. It would be better if the boiling temperature were higher to prevent the loss of antioxidant value. The medicinal chemistry-related parameters, namely QED, GASA, NP score, Lipinski's rule, colloidal aggregators, reactive compounds, and promiscuous compounds, were analysed, and these values were predicted to be at acceptable levels. The absorption characteristics of ethyl vanillin showed that it had low permeability with a PAMPA value of 0.26. The HIA value and F50% value showed an average bioavailability. The CLplasma was determined to be moderate, and $T_{1/2}$ was predicted to be short. In this respect, these parameters are also deemed reasonable. The predicted value of FDAMDD was recorded as 0.25 mmol/kg body weight/day. The values for skin sensitisation and carcinogenicity were evaluated as intermediate toxicity—human hepatotoxicity, drug-induced nephrotoxicity, and Hek293 cytotoxicity, potentially indicating low toxicity. Furthermore, no potential acute toxicity effects were found for ethyl vanillin.

## Conclusion

Several synthetic and natural compounds are used as preservatives to prevent spoilage, growth of pathogenic bacteria, and rancidity, among other purposes, or to flavour food products, thereby preventing economic losses in the food industry. Ethyl vanillin, a synthetic vanilla derivative, is one of the flavouring additives used mainly in bakery products or chocolate, etc. In the present study, we demonstrated that ethyl vanillin has potential antibacterial and antibiofilm efficacy against *B. cereus* and *E. coli* O157:H7. These two foodborne pathogens can cause significant financial losses or pose a threat to public health. The antibacterial and antibiofilm activities of ethyl vanillin were found to be higher even at lower concentrations, especially against *B. cereus* when compared to *E. coli* O157:H7. Antioxidant activity and total phenolic content were also determined to be high for ethyl vanillin. Ethyl vanillin was found to be acceptable for many features according to ADMET analysis. In the food industry, ADMETlab 3.0 offers a rapid and labour-saving alternative for analysing specific properties of food compounds. Overall, these results suggest that the benefits of ethyl vanillin are not limited to flavour/aroma, but may also have antibacterial, antibiofilm and antioxidant potential in food samples. As the difficulty and high cost of obtaining vanilla are drawbacks to its use, further research into the use of a derivative of ethyl vanillin could be beneficial to the food sector. Based on these results, we suggest that ethyl vanillin may have a positive effect on shelf life. In this context, the protective activity of ethyl vanillin can be tested in different food products. Considering that foodborne infection/intoxication due to bacterial growth is a common food hygiene problem in the food sector, or problems such as biofilm formation on equipment in the food industry, active films that provide good shelf life can be prepared by incorporating ethyl vanillin directly as an active component or incorporated into other components with synergistic effects.

**Compliance with Ethical Standards**

**Conflict of interest:** The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

**Ethics committee approval:** The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

**Data availability:** The data will be made available upon request by the author(s).

**Funding:** -

**Acknowledgements:** We are grateful to Eurofins Scientific Food Analysis Laboratory for assistance in obtaining food pathogenic bacteria.

**Disclosure:** -

## References

**Anand, S., Singh, D., Avadhanula, M., & Marka, S. (2014)**. Development and control of bacterial biofilms on dairy processing membranes. *Comprehensive Reviews in Food Science and Food Safety*, 13(1), 18-33. https://doi.org/10.1111/1541-4337.12048

**Arya, S.S., Rookes, J.E., Cahill, D.M., & Lenka, S.K. (2021).** Vanillin: A review on the therapeutic prospects of a popular flavouring molecule. *Advances in traditional medicine*, 1-17. https://doi.org/10.1007/s13596-020-00531-w

**Banerjee, G., & Chattopadhyay, P. (2019).** Vanillin biotechnology: the perspectives and future. *Journal of the Science of Food and Agriculture,* 99(2), 499-506. https://doi.org/10.1002/jsfa.9303

**Cava-Roda, R.M., Taboada-Rodríguez, A., Valverde-Franco, M.T., & Marín-Iniesta, F. (2012).** Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *Listeria monocytogenes* and *Escherichia coli* O157: H7 in milk. *Food and Bioprocess Technology,* 5, 2120-2131. https://doi.org/10.1007/s11947-010-0484-4

**Chen, P., Liu, Y., Li, C., Hua, S., Sun, C., & Huang, L. (2023).** Antibacterial mechanism of vanillin against *Escherichia coli* O157: H7. *Heliyon*, 9(9). https://doi.org/10.1016/j.heliyon.2023.e19280

**Choe, E., & Min, D.B. (2006).** Chemistry and reactions of reactive oxygen species in foods. *Critical reviews in food science and nutrition*, 46(1), 1-22. https://doi.org/10.1080/10408390500455474

**Clarke, G, Ting, K.N., Wiart, C, Fry, J. (2013).** The high correlation between 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential, and total phenolic content indicates redundancy in the use of all three assays to screen for antioxidant activity in extracts of plants from the Malaysian rainforest. *Antioxidants,* 2, 1–10. https://doi.org/10.3390/antiox2010001

**de Jager, L.S., Perfetti, G.A., & Diachenko, G.W. (2007)**. Determination of coumarin, vanillin, and ethyl vanillin in vanilla extract products: liquid chromatography mass spectrometry method development and validation studies. *Journal of chromatography A*, 1145(1-2), 83-88. https://doi.org/10.1016/j.chroma.2007.01.039

**Fache, M., Boutevin, B., & Caillol, S. (2016)**. Vanillin production from lignin and its use as a renewable chemical. *ACS sustainable chemistry & engineering*, 4(1), 35-46. https://doi.org/10.1021/acssuschemeng.5b01344

**Fitzgerald, D.J., Stratford, M., Gasson, M.J., Ueckert, J., Bos, A., & Narbad, A. (2004).** Mode of antimicrobial action of vanillin against *Escherichia coli, Lactobacillus plantarum* and *Listeria innocua*. *Journal of applied microbiology*, 97(1), 104-113. https://doi.org/10.1111/j.1365-2672.2004.02275.x

**Future Market Insights (2024).** Vanillin Report. Vanillin Market Snapshot from 2024 to 2034. https://www.futuremarketinsights.com/reports/vanillin-market Accessed December 23, 2024.

**Grudlewska-Buda, K., Bauza-Kaszewska, J., Wiktorczyk-Kapischke, N., Budzyńska, A., Gospodarek-Komkowska, E., & Skowron, K. (2023).** Antibiotic Resistance in selected emerging bacterial foodborne Pathogens—An issue of concern?. *Antibiotics*, *12*(5), 880. https://doi.org/10.3390/antibiotics12050880

**Hassanain, N.A., Hassanain, M.A., Ahmed, W.M., Shaapan, R.M., Barakat, A.M., & El-Fadaly, H.A. (2013).** Public health importance of foodborne pathogens. *World Journal of Medical Sciences* 9(4), 208-222.

**Huang, Y., Flint, S. H., & Palmer, J.S. (2020).** *Bacillus cereus* spores and toxins–The potential role of biofilms. *Food microbiology*, 90, 103493. https://doi.org/10.1016/j.fm.2020.103493

**Kadir, N.A., Naher, L., & Sidek, N. (2019).** Economical important phytopathogenic diseases in *Vanilla planifolia*: A review paper. *Journal of Tropical Resources and Sustainable Science* (JTRSS), 7(2), 77-82.
https://doi.org/10.47253/jtrss.v7i2.513

**Karameşe, M., & Dicle, Y. (2022).** The antibacterial and antibiofilm activities of resveratrol on Gram-positive and Gram-negative bacteria. *Kafkas Journal of Medical Sciences*, 12(3), 201-206.
https://doi.org/10.5505/kjms.2022.76743

**Kumar, R., Sharma, P.K., & Mishra, P.S. (2012).** Vanillin derivatives showing various biological activities. *ChemInform*, 43(28).

**Li, T., He, B., Mei, Y., Wang, D., Sun, X., & Li, J. (2019).** Inhibitory effect of vanillin on the virulence factors and biofilm formation of *Hafnia alvei*. *LWT,* 102, 223-229.
https://doi.org/10.1016/j.lwt.2018.12.038

**Liu, X., Yao, H., Zhao, X., & Ge, C. (2023).** Biofilm formation and control of foodborne pathogenic bacteria. *Molecules*, 28(6), 2432.
https://doi.org/10.3390/molecules28062432

**Lu, J., Hu, X., & Ren, L. (2022).** Biofilm control strategies in food industry: Inhibition and utilization. *Trends in Food Science & Technology*, 123, 103-113.
https://doi.org/10.1016/j.tifs.2022.03.007

**Mogana, R., Adhikari, A., Tzar, M.N., Ramliza, R., & Wiart, C.J.B.C M. (2020).** Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. *BMC Complementary Medicine and Therapies*, 20, 1-11.
https://doi.org/10.1186/s12906-020-2837-5

**Morlock, G.E., Busso, M., Tomeba, S., & Sighicelli, A. (2021).** Effect-directed profiling of 32 vanilla products, characterization of multi-potent compounds and quantification of vanillin and ethylvanillin. *Journal of Chromatography A*, 1652, 462377.
https://doi.org/10.1016/j.chroma.2021.462377

**Nan, Y., Rodas-Gonzalez, A., Stanford, K., Nadon, C., Yang, X., McAllister, T., & Narváez-Bravo, C. (2022).** Formation and transfer of multi-species biofilms containing *E. coli* O103: H2 on food contact surfaces to beef. *Frontiers in Microbiology*, 13, 863778.
https://doi.org/10.3389/fmicb.2022.863778

**Ngarmsak, M., Delaquis, P., Toivonen, P., Ngarmsak, T., Ooraikul, B., & Mazza, G. (2006).** Antimicrobial activity of vanillin against spoilage microorganisms in stored fresh-cut mangoes. *Journal of Food Protection*, 69(7), 1724-1727.
https://doi.org/10.4315/0362-028X-69.7.1724

**Olaimat, A.N., Ababneh, A.M., Al-Holy, M., Al-Nabulsi, A., Osaili, T., Abughoush, M., ... & Holley, R.A. (2024).** A Review of Bacterial Biofilm Components and Formation, Detection Methods, and Their Prevention and Control on Food Contact Surfaces. *Microbiology Research*, 15(4), 1973-1992.
https://doi.org/10.3390/microbiolres15040132

**Oluwarinde, B.O., Ajose, D.J., Abolarinwa, T.O., Montso, P.K., Du Preez, I., Njom, H.A., & Ateba, C.N. (2023)**. Safety properties of *Escherichia coli* O157:H7 specific bacteriophages: recent advances for Food Safety. *Foods*, 12(21), 3989.
https://doi.org/10.3390/foods12213989.

**Ombarak, R.A., Hinenoya, A., Awasthi, S.P., Iguchi, A., Shima, A., Elbagory, A.R.M., et al. (2016)**. Prevalence and pathogenic potential of *Escherichia coli* isolates from raw milk and raw milk cheese in Egypt. *International Journal of Food Microbiology*, 221, 69-76.
https://doi.org/10.1016/j.ijfoodmicro.2016.01.009

**Ooka, M., Sakamuru, S., Zhao, J., Qu, Y., Fang, Y., Tao, D., ... & Xia, M. (2024).** Use of Tox21 screening data to profile PFAS bioactivities on nuclear receptors, cellular stress pathways, and cytochrome P450 enzymes. *Journal of Hazardous Materials*, 473, 134642.
https://doi.org/10.1016/j.jhazmat.2024.134642

**Orhan-Yanıkan, E., da Silva-Janeiro, S., Ruiz-Rico, M., Jiménez-Belenguer, A.I., Ayhan, K., & Barat, J.M. (2019).** Essential oils compounds as antimicrobial and antibiofilm agents against strains present in the meat industry. *Food Control*, 101, 29-38.

https://doi.org/10.1016/j.foodcont.2019.02.035

**Öztürk, F.Y., Darcan, C., & Kariptaş, E. (2023).** The determination, monitoring, molecular mechanisms and formation of biofilm in *E. coli*. *Brazilian Journal of Microbiology*, 54(1), 259-277.
https://doi.org/10.1007/s42770-022-00895-y

**Peng, J., Wei, M., Hu, Y., Yang, Y., Guo, Y., & Zhang, F. (2019).** Simultaneous determination of maltol, ethyl maltol, vanillin, and ethyl vanillin in foods by isotope dilution headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Food Analytical Methods*, 12, 1725-1735.
https://doi.org/10.1007/s12161-019-01518-3

**Ponnusamy, K., Paul, D., & Kweon, J.H. (2009).** Inhibition of quorum sensing mechanism and *Aeromonas hydrophila* biofilm formation by vanillin. *Environmental Engineering Science*, 26(8), 1359-1363.
https://doi.org/10.1089/ees.2008.0415

**Retnosari, R., Rachman, I.B., Sutrisno, S, Sari, M.E., Sukarianingsih, D., Rukayadi, Y. (2021).** The antibacterial activity of vanillin derivative compounds. *In: AIP Conference Proceedings*, Vol. 2349, Surabaya, Indonesia.
https://doi.org/10.1063/5.0051523

**Tai, A., Sawano, T., & Yazama, F. (2011).** Antioxidant properties of ethyl vanillin *in vitro* and *in vivo*. *Bioscience, Biotechnology, and Biochemistry*, 75(12), 2346-2350.
https://doi.org/10.1271/bbb.110524

**Tai, A., Sawano, T., Yazama, F., & Ito, H. (2011).** Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1810(2), 170-177.
https://doi.org/10.1016/j.bbagen.2010.11.004

**Thaweboon, S., Thaweboon, B., & Kaypetch, R. (2017)**. In vitro inhibitory effect of vanillin on *Candida* biofilm. *In Materials Science Forum* (Vol. 909, pp. 177-181). Trans Tech Publications Ltd.
https://doi.org/10.4028/www.scientific.net/MSF.909.177

**Vaghasiya, Y. K., Nair, R., Soni, M., Baluja, S., & Shanda, S. (2004).** Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine. *Journal of the Serbian Chemical Society*, 69(12), 991-998.
https://doi.org/10.2298/JSC0412991V

**Wang, Z., Zeng, G., Wei, X., Ding, B., Huang, C., & Xu, B. (2016).** Determination of vanillin and ethyl-vanillin in milk powder by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Food Analytical Methods*, 9, 3360-3366.
https://doi.org/10.1007/s12161-016-0520-8

**WHO (2022).** Food safety.
https://www.who.int/news-room/fact-sheets/detail/food-safety (Accessed 21 December 2024)

**Yang, H., Dong, Y., Du, H., Shi, H., Peng, Y., & Li, X. (2011).** Antioxidant compounds from propolis collected in Anhui, China. *Molecules*, 16(4), 3444-3455.
https://doi.org/10.3390/molecules16043444

**Yemis, G.P., Pagotto, F., Bach, S., & Delaquis, P. (2011).** Effect of vanillin, ethyl vanillin, and vanillic acid on the growth and heat resistance of *Cronobacter* species. *Journal of Food Protection*, 74(12), 2062-2069.
https://doi.org/10.4315/0362-028X.JFP-11-230

**Yin, W., Wang, Y., Liu, L., & He, J. (2019).** Biofilms: the microbial "protective clothing" in extreme environments. *International Journal of Molecular Sciences*, 20(14), 3423.
https://doi.org/10.3390/ijms20143423