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Hesperidin methyl chalcone, a citrus flavonoid, inhibits *Aeromonas hydrophila* infection mediated by quorum sensing

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Abstract

Plant-derived phytocompounds are effective in treating a variety of ailments and disorders, the most common of which are bacterial infections in humans, which are a major public health concern. Flavonoids, one of the groups of phytocompounds, are known to have significant antimicrobial and anti-infective properties. Hence, the current study investigates the efficacy of the citrus flavonoid hesperidin methylchalcone (HMC) in addressing this major issue. The results of this study indicate that the anti-quorum sensing (anti-QS) action against Aeromonas hydrophila infections is exhibited with a decrease in biofilm development and virulence factors production through *in vitro* and in silico analyses. In addition, the qPCR findings indicate that HMC has antivirulence action on *A. hydrophila* by reducing the expression of QS-related virulence genes, including *ahyR, ahyB, ahh1, aerA,* and lip. Interestingly, HMC significantly rescued the *A. hydrophila*-infected zebrafish by reducing the internal colonization, demonstrating the *in vivo* anti-infective potential of HMC against *A. hydrophila* infection. Based on these results, this study recommends that HMC could be employed as a possible therapeutic agent to treat *A. hydrophila*-related infections in humans.

Introduction

Over the last few decades, medicinal systems from various sources have encouraged the use of edible substances, especially those obtained from plants to prevent and cure human diseases [1]. Plant-derived foods containing such phytochemicals are generally low in toxicity and target several cell signaling pathways involved in the infectious process [2]. Amid the discovery of several plant-based medications, novel bioactive substances need to be explored that can expand the range of conventional antibiotics and help identify potentially important medicinal remedies [3]. In vitro and *in vivo* models have been extensively used to study the bioactive properties of phytochemicals, which yields valuable insights into structure-function interactions that may be responsible for disease risk reductions [4]. Generally, phytochemicals have been grouped into six main classes based on their chemical compositions and properties, which include carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogen-containing molecules. Under each class, subcategories are formed by further division depending on biogenesis or biosynthetic sources [5]. Flavonoids are one such subcategory of the phenolic family of compounds. They are natural compounds widely distributed in fruits and vegetables, known to constitute one of the largest groups of natural products known [6]. It shows diverse biological properties, including non-hepatotoxic, antimicrobial, anti-tumor, antioxidant, anti-allergic, anti-inflammatory, and analgesic properties, which could be used in various medicinal applications [7]. Hence, this study is aimed at evaluating the efficacy of HMC, a water-soluble flavanone that is capable of preventing quorum sensing (QS) mediated biofilm formation and the production of virulence factors by Aeromonas hydrophila.

A. hydrophila is a rod-shaped, gram-negative bacterium present predominantly in drinking water, wastewater, sewage, and food [8]. It causes infection in both animals and humans. In fish, *A. hydrophila* causes infections like motile aeromonad septicaemia and hemorrhagic septicaemia, which result in fin rot, tail rot, scale protrusion disease, and ulcerative diseases [9,10]. Eventually, these infections of *A. hydrophila* are transmitted from diseased fish to humans by polluted water and by consuming raw seafood. As a result, they exhibit a range of virulence determinants linked to human diseases such as gastroenteritis, soft tissue and muscle infections, septicaemia, and skin diseases, and they are increasingly regarded as enteric pathogens which are of major public health concern [11]. It has also been reported that, in the field of reconstructive microsurgery, the use of surgical leeches (*Hirudo medicinalis*) for the treatment of postoperative venous congestion carries a high risk of acute and delayed infection with *A. hydrophila* [12]. Considering all these data, *A. hydrophila* has gained much more importance over the last decade as a food-borne zoonotic pathogen. As far as treatments are concerned, antibiotics like oxytetracycline, doxycycline, florfenicol, ciprofloxacin, norfloxacin, amoxicillin, ampicillin, cephradine, erythromycin, and sulfamethoxazole-trimethoprim are being used to treat *Aeromonas* infections [13]. The use of antibiotics indiscriminately for an extended period to treat bacterial infections exerts selection pressure on a bacterium which can result in the development of antibiotic-resistant bacterial strains. Owing to the emergence of multiresistant strains of *Aeromonas,* the treatment that has been given so far has become complicated [14,15]. With the increase of antibiotic-resistant *Aeromonas* strains in aquatic environments, an alternative and effective therapeutic approach for combating these infections is deemed necessary [14]. On that account, understanding bacterial pathogenicity begins with determining the bacterial virulence factors. Multiple virulence factors interact separately or in combination to induce disease in susceptible hosts, and pathogenic

bacteria generate a variety of substances that are toxic to host cells either directly or indirectly [16,17]. One of the solutions for disrupting these toxic substances is to target the quorum sensing (QS) system, which is also known as the bacterial intercellular communication system. This QS system is essential to produce virulence factors and the formation of biofilms in bacterial pathogens. *A. hydrophila* is an opportunistic pathogen that uses N-acyl homoserine lactones in its *AhyI/R* QS signaling pathway (AHLs). These AHLs play a significant role in biofilm formation as well as the development of virulence factors like protease, lipase, and hemolysin [18]. Interference in such a QS system leads to the inhibition of virulence factor production and biofilm formation by Aeromonas spp [19]. Hence, the current research work aims to investigate the role of HMC against A. hydrophila infections through *in vitro*, in silico, and *in vivo analysis*.

Section snippets

Bacterial strain and culture conditions

In this work, the reference strain A. hydrophila obtained from the Microbial Type Culture Collection (MTCC 1739) was used. Generally, the culture to be tested is inoculated in Luria-Bertani (LB) medium at 30°C overnight. For experimental purposes, the test isolate was subcultured in LB medium and adjusted to OD 0.4at 600nm (1×10^8CFU/ml). For long-term storage and maintenance of the test culture, glycerol stocks (60%) were prepared and stored at deep freezing conditions (−80°C).…

Compound preparation

HMC ($C_{29}H_{36}$…

HMC inhibits biofilm formation in *A. hydrophila*

In general, a biofilm is an aggregated growth of microorganisms, wherein the cells encase themselves in a self-produced extracellular polymeric substance, which plays an important role in their pathogenesis and exhibits resistance to antibiotics [37]. The obtained results of biofilm assay demonstrated that HMC effectively reduced *A. hydrophila* biofilm formation without inhibiting its growth. The percentage of inhibition was found to be 74% and 75% at 600μg/ml and 800μg/ml concentrations,…

Conclusion

To summarize, the purpose of this study was to assess the antibiofilm and anti-infective potential of HMC to treat *A. hydrophila* infection. The *in vitro* results revealed that HMC considerably reduced the biofilm formation and virulence factors production, including protease, lipase, and hemolysin in *A. hydrophila*. In addition, HMC greatly reduced the EPS production, hydrophobicity, and swimming motility of *A. hydrophila* at BIC treatment. Interestingly, the docking and molecular dynamics results …

Declaration of competing interest

The authors have no conflict of interest to declare.…

CRediT authorship contribution statement

Prithiviraj Swasthikka Roshni: Formal analysis, Data curation, Methodology, Software, Validation, Writing – original draft. **Rajaiah Alexpandi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Gurusamy Abirami:** Validation, Formal analysis, Data curation. **Ravindran Durgadevi:** Data curation, Formal analysis. **Yurong Cai:** Data curation, Writing – review & editing. **Ponnuchamy Kumar:** Data curation, Writing…

Declaration of competing interestDoCI

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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