Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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To cite this article: Jianteng Wei, Shuxian Wang, Dong Pei, Liangjing Qu, Ya Li, Jianjun Chen, Duolong Di & Kun Gao (2018) Antibacterial Activity of Hydroxytyrosol Acetate from Olive Leaves (*Olea Europaea* L.), Natural Product Research, 32:16, 1967-1970, DOI: 10.1080/14786419.2017.1356830

To link to this article: https://doi.org/10.1080/14786419.2017.1356830

NATURAL PRODUCT RESEARCH
2018, VOL. 32, NO. 16, 1967–1970
https://doi.org/10.1080/14786419.2017.1356830

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Antibacterial Activity of Hydroxytyrosol Acetate from Olive Leaves (*Olea Europaea* L.)

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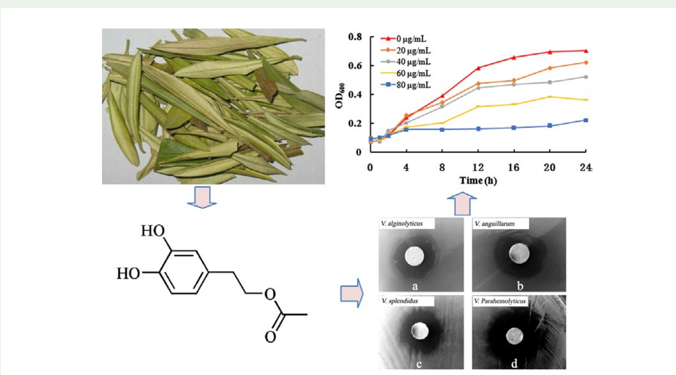
ABSTRACT

Vibrio spp. are pathogens of many bacterial diseases which have caused great economic losses in marine aquaculture. The strategy of alternative medical treatment that is utilised by herbalists has expanded in the past decade. The aim of our study is to discover the antibacterial molecules against *Vibrio* spp. Bacterial growth inhibition, membrane permeabilisation assessment and DNA interaction assays, as well as agarose gel electrophoresis, were employed to elucidate the antibacterial activity of hydroxytyrosol acetate. Results showed that hydroxytyrosol acetate had antibacterial activity against *Vibrio* spp. and it played the role via increasing bacterial membrane permeabilisation. The DNA interaction assay and agarose gel electrophoresis revealed that hydroxytyrosol acetate interacted with DNA. Hydroxytyrosol acetate enhanced the fluorescent intensity of DNA binding molecules and mediated supercoiled DNA relaxation. The present study provides more evidence that hydroxytyrosol acetate is a novel antibacterial candidate against *Vibrio* spp.



ARTICLE HISTORY
Received 25 February 2017
Accepted 22 June 2017

KEYWORDS
Hydroxytyrosol acetate; olive leaves; antibacterial activity; DNA interaction

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1356830.

1. Introduction

Vibrio spp., Gram-negative bacteria, are the pathogens of many bacterial diseases and these diseases such as septicemia of pseudosciaena crocea and red appendages disease of prawn; red appendages disease of prawn have caused great economic losses in marine aquaculture. Although the use of antibiotic has partially solved the problem of *Vibrio* infections, it has raised other problems, such as drug resistance and environmental contamination and food safety. To overcome these problems, it is necessary to discover safer and more effective antibacterial drugs with minimal risks to human and environmental health.

Olive leaves (*Olea europaea* L.) are used in the folk medicine of the Mediterranean region to treat fever and other diseases (El and Karakaya 2009). It is reported that olive leaves have antioxidant, hypoglycemic, antihypertensive, antimicrobial, anti-tumoral, antiatherosclerotic and antiviral, including anti-HIV, properties (Rahmanian et al. 2015). Oleuropein and its derivatives, such as hydroxytyrosol and tyrosol, may have antimicrobial effects (Báidez et al. 2007). Kalaiselvan et al. (2015) found that olive oil and its phenolic constituent tyrosol could attenuate dioxin-induced toxicity via an antioxidant-dependent mechanism. (Brahmi et al. 2012) reported that the antimicrobial activity of olive leaves is related to their terpene content; terpenes act by disrupting cell membranes. (Sudjana et al. 2009) found that olive leaf extracts selectively reduce levels of *Campylobacter jejuni* and *Helicobacter pylori* to regulate the composition of gastric flora. Although more and more researchers have recently paid attention to the antibacterial activity of olive leaves, the material basis and mechanism of action of this activity are not fully understood.

The aim of our study is to discover the antibacterial molecules that are responsible for the antibacterial activity of olive leaves, as well as to investigate the mechanisms of action of these compounds. In our screening programme, we found that hydroxytyrosol acetate (Figure S1) has significant antibacterial activity, which has not been reported in previous studies.

2. Results and discussions

The strategy of alternative medical treatment that is utilised by herbalists has expanded in the past decade. The antibacterial activities of many plants have been reported (Tiwari et al. 2009) and are attributed to compounds like butanol, unsaturated long-chain aldehydes, peptides, essential oils, alkaloids and phenols (Zielińska and Matkowski 2014). These compounds have potential therapeutic applications against *Vibrio* diseases (Samad et al. 2014; Wu et al. 2015).

The assay revealed that hydroxytyrosol acetate from olive leaves has antibacterial activity against *Vibrio* spp. (Figure S2, Table S1). This antibacterial activity of hydroxytyrosol acetate is a novel finding. The MIC and MBC of hydroxytyrosol acetate against *V. parahemolyticus* were 39 μg/mL and 78 μg/mL, respectively (Table S2). (Bisignano et al. 1999) reported that hydroxytyrosol and oleuropein from olive leaves had antibacterial activity in the previous study.

Hydroxytyrosol acetate could inhibit the growth of *V. parahemolyticus*. Treatment with 80 μg/mL hydroxytyrosol acetate almost completely stopped bacterial growth during incubation (Figure S3). The PI staining assay results suggested that hydroxytyrosol acetate increases membrane permeabilisation in *V. parahemolyticus* (Table S3). Loss of membrane integrity has also been reported in several antibacterial agents, including 2R, 3R-Dihydromyricetin and Microcin E492. These compounds usually lead to the leakage of cellular constituents, such as soluble proteins and carbohydrates (Destoumieux-Garzón et al. 2003; Wu et al. 2017). Our study provides more evidence that affecting the integrity of the cell membrane is one of the main mechanisms for antibacterial agents.

Many antibacterial agents interact with DNA molecules (Bolhuis and Aldrich-Wright 2014). The absorption spectra are the most common means to examine the interaction between antibacterial agents and DNA. The UV–vis spectrum of ctDNA showed maximum absorption at 260 nm, whereas hydroxytyrosol acetate alone had relatively low absorption. However, when hydroxytyrosol acetate was added to the ctDNA solution, the UV–vis spectrum changed, which indicated that hydroxytyrosol acetate and ctDNA interacted with each other (Figure S4). DAPI, AO and Hoechst3325, DNA binding agents, can interact with DNA molecules (Nafisi et al. 2007; Palchaudhuri and Hergenrother 2007; Banerjee and Pal 2008). The assay results showed that hydroxytyrosol acetate enhanced the fluorescence intensities of the DAPI–DNA complex, the AO–DNA complex and Hoechst33258–DNA complex (Figure S4). Moreover, the results of the supercoiled DNA relaxation assay suggested that hydroxytyrosol acetate mediates the relaxation of supercoiled DNA (Figure S5). In our previous report, we found that BDDE might display antibacterial activity by interacting with DNA. The compound could change the structure of DNA, and then affect its biological function by interacting with DNA (Liu et al. 2012). Our study reveals that interacting with DNA is also an important mechanism for antibacterial agents.

3. Conclusions

In conclusion, the present study reveals that hydroxytyrosol acetate displays inhibition against *Vibrio* spp. Hydroxytyrosol acetate inhibits the growth of *V. parahemolyticus*, and destroys the integrity of the bacterial membrane. Hydroxytyrosol acetate interacts with DNA and both the membrane disruption effect and the DNA binding activities may contribute to the antibacterial activity of hydroxytyrosol acetate. Therefore, hydroxytyrosol acetate may serve as a novel antibacterial candidate.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Sciences Foundation of China under [grant number 21505143]; Qingdao Science and Technology Project under [grant number 15-8-2-6-hy]; and the STS Program of the Chinese Academy of Sciences under [grant number KFJ-SW-STS-146].

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