

RESEARCH NOTE

Open Access



Antibacterial activity of thymoquinone derivative

Mohammad Yasin Mohammad^{1*}, Haroon M. Haniffa² and M. Iqbal Choudhary³

Abstract

Natural products such as terpenoidal compounds have been extremely tested against pathogenic bacteria. Researches are frequently carried out to find out new natural, semisynthetic and synthetic antibacterial agents due to problems of resistance. Thymoquinone derivative was obtained in our previous study and the current research is a continuation. The antibacterial activity of a monoterpene; thymoquinone derivative, 5-isopropyl-2-methyl-oxepine-1-one (**1**) has been evaluated for the first time by following the Agar cup bioassay method employed. The bacterial strains used in this study were *Escherichia coli* and *Bacillus subtilis*. Compound **1** showed moderate activity against Gram-positive organism; *B. subtilis* and good activity against Gram-negative species; *E. coli* with zones of inhibition (ZOI) 10.0 ± 0.2 mm and 11.0 ± 0.2 mm against *E. coli* and *B. subtilis*, respectively, and in comparison with antibiotic, imipenem. The zones of inhibition were calculated as the mean of the triplicate. The antibacterial activity of thymoquinone derivative **1** could be explained by the presence of unsaturated lactone.

Keywords Monoterpene, Pathogenic bacteria, *Bacillus subtilis*, *Escherichia coli*

Introduction

Thymoquinone (2-Isopropyl-5-methyl-[1,4]benzoquinone, $C_{10}H_{12}O_2$), a monoterpene isolated from the seeds of *Nigella sativa*, has been shown to have antioxidant [1], analgesic [2], anticonvulsant effects [3] and antibacterial activity [4].

The derivative of thymoquinone, 5-isopropyl-2-methyl-oxepine-1-one (**1**), was obtained for the first time from our previous work on biotransformation of thymoquinone using *Aspergillus niger* [5], and the current study is a continuation to our research.

In continuation of the biotransformation study on thymoquinone [5], we reported the antibacterial activity of thymoquinone derivative **1** on bacterial strains; *Escherichia coli* and *Bacillus subtilis*.

Antibacterial resistance which occurs naturally, is one of the biggest problems to global health. It can affect any patient, of any age, in any country [6]. In addition, there are problems with the patient staying in the hospital for a longer period of time, high treatment costs, and increased mortality rate [7]. Antibacterial drug resistance by *E. coli* and *B. subtilis* is one of the challenges facing the medical sector [8, 9], so in this study, we evaluated the antibacterial activity against both *E. coli* and *B. subtilis* in order to find out a new lead as a starting point for developing it as antibacterial drug.

Experimental

Antibacterial test experiment

The standard strains of bacteria used for this study were *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 12,022. The bacteria were grown on Muller Hinton II

*Correspondence:

Mohammad Yasin Mohammad
myasin@aut.edu.jo; mhm17feb@hotmail.com

¹ Faculty of Pharmacy, Aqaba University of Technology, Aqaba 11191, Jordan

² Department of Chemical Sciences, Faculty of Applied Sciences, South Eastern University, Oluvil, Sri Lanka

³ H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

agar (Oxoid) and incubated at 35°C while slants were prepared and stored at -4°C for further studies. Single colony of the test organism was incubated in to nutrient broth for 18 h and diluted 10⁵ folds to obtain approximately 10⁶ colony forming units (cfu) per mL of culture suspension. 100 µL of bacterial suspension was transferred into sterile soft agar tube and properly missed. This was aseptically transmitted into sterile solidified nutrient agar plate to form lawn and gently swirl to ensure even distribution of the test culture and allowed to properly solidify. Sterile cork borer was used to make wells on solidified medium and properly labeled. 1 mg/1 mL of test compound was dissolved in DMSO with 100 µL of test compound in respective well plate according to bacterial culture in triplicates. Reference antibiotic drug and DMSO were added to separate wells as positive and negative control. The plates were allowed to rest for 30 min for proper diffusion prior to incubation at 37°C for 24 h. The zones of inhibition were measured in mm with transparent ruler and the average was calculated as the mean of the triplicate.

Results and discussion

The antibacterial activity of thymoquinone derivative, 5-isopropyl-2-methyloxepine-1-one (**1**) has been evaluated for the first time (Table 1) by following the Agar cup bioassay method employed [10]. Compound **1** has shown zones of inhibition (ZOI) 10.0±0.2 mm and 11.0±0.2 mm against *Escherichia coli* and *Bacillus subtilis*, respectively, and in comparison with antibiotic, imipenem. The zones of inhibition were calculated as the mean of the triplicate.

The antibacterial activity of compound **1** could be explained by the presence of unsaturated lactone [11], since natural products of lactone functionality including

terpenoidal lactones showed good anti-bacterial activities against various Gram-positive and Gram-negative organisms [12]. In support of this, for instance, the sesquiterpenoidal bicyclic lactone; costunolide exhibited potent activities against *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* with MIC 12.5 mg/L and 0.5 mg/mL, respectively [13, 14]. Similarly, the sesquiterpenoidal lactone; partenolide showed potent inhibitions of bacterial growth against *Staphylococcus aureus* and *P. aeruginosa* with MIC 0.08 mg/mL and 0.531 mg/mL, respectively [14].

The antibacterial activities of compound **1** against both *E. coli* and *B. subtilis* were good and not weak compared to the antibiotic; imipenem, and this explains the importance of its lactone functionality to give good activities. However, compound **1** is a lead compound and might be further developed to become an antibacterial agent.

Limitations

- Insufficient sample amount for tests on other bacterial strains.
- Limited available bacterial strains in our research laboratories.
- Lack of available techniques to evaluate the MIC in this study.

Conclusion

Compound **1** is a lead compound that showed significant zone of inhibition against both Gram-positive organism; *Bacillus subtilis* and Gram-negative species; *Escherichia coli*, and in comparison with antibiotic; imipenem. In conclusion, metabolite **1** can be further developed to be an antibacterial drug.

Acknowledgements

We acknowledge Aqaba University of Technology, Aqaba, Jordan for support.

Author contributions

MYM has written the results and discussion and the abstract parts. HMH has performed experimental part. MIC has checked the abstract and introduction parts.

Funding

None.

Availability of data and materials

Not applicable.

Declarations

The authors whose names are listed certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

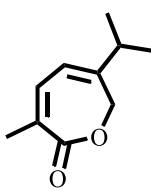
Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Table 1 Inhibition zone diameter (mm) of compound **1***

Compound (100 µg/mL)	Structure	Gram positive <i>Bacillus subtilis</i>	Gram negative <i>Escherichia coli</i>
1		11.0±0.2	10.0±0.2
DMSO	-	-	-
Imipenem	-	29.0±0.1	24.0±0.2

Compound with a concentration of 100 µg/mL was tested against the organisms Results were recorded after 24 h of treatment and inhibitory zone diameters were measured in mm

-: no inhibition

*Values are mean of triplicate

Competing interests

The authors declare no competing interests.

Received: 20 December 2022 Accepted: 21 September 2023

Published online: 06 October 2023

References

- Houghton PJ, Zarka R, De Las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* 1995;61(1):33–6.
- Abdel-Fattah AM, Matsumoto K, Watanabe H. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. *Eur J Pharmacol.* 2000;400(1):89–97.
- Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. *Phytomedicine: Int J Phytother Phytopharmacol.* 2004;11(1):56–64.
- Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. *BMC Complement Altern Med.* 2011;11: 29.
- Mohammad MY, Shakya A, Al-Bakain R, Haroon MH, Choudhary MI. New monoterpeneoid by biotransformation of thymoquinone using *Aspergillus niger*. *Bioorg Chem.* 2018;80:212–5.
- Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. *PLoS One.* 2017;12: e0189621.
- Shrestha P, Cooper BS, Coast J, Oppong R, Thuy NDT, Phodha T, Celhary O, Guerin PJ, Wertheim H, Lubell Y. Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. *Antimicrob Resist Infect Control.* 2018;7(1):98.
- Puvača N, de Llanos Frutos R. Antimicrobial resistance in *Escherichia coli* strains isolated from humans and pet animals. *Antibiotics.* 2021;10: 69.
- Adimpong DB, Sørensen KI, Thorsen L, Stuer-Lauridsen B, Abdelgadir WS, Nielsen DS, Derx PM, Jespersen L. Antimicrobial susceptibility of *Bacillus* strains isolated from primary starters for african traditional bread production and characterization of the bacitracin operon and bacitracin biosynthesis. *Appl Environ Microbiol.* 2012;78(22):7903–14.
- Jennessen J, Nielsen KF, Houbraken J, Lyhne EK, Schnürer J, Frisvad J, Samson RA. Secondary metabolite and mycotoxin production by the *Rhizopus microspores* group. *J Agric Food Chem.* 2005;53(55):1833–40.
- Winska K, Grabarczyk M, Maczka W, Zarowska B, Maciejewska G, Anil M. Antimicrobial activity of new bicyclic lactones with three or four methyl groups obtained both synthetically and biosynthetically. *J Saudi Chem Soc.* 2018;22(3):363–71.
- Mazur M, Masłowiec D. Antimicrobial activity of lactones. *Antibiotics.* 2022;11:1–27.
- Kim DY, Choi BY. Costunolide—a bioactive sesquiterpene lactone with diverse therapeutic potential. *Int J Mol Sci.* 2019;20: 2926.
- Häkkinen ST, Soković M, Nohynek L, Čirić A, Ivanov M, Stojković D, Tsitko I, Matos M, Baixinho JP, Ivasiv V, et al. Chicory extracts and sesquiterpene lactones show potent activity against bacterial and fungal pathogens. *Pharmaceuticals.* 2021;14: 941.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

