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Determination of Minimum Inhibitory Concentrations of 4,4'-diacetyldiphenylselenide in Antibacterial Activity

Nadjah Belattar[1*], Youcef Mechehoud[1], Samir Benayache[1], Kaddour Benlabed[2], Narimane Segueni[3] and Fadila Benayache[1]

[1]VAREN Laboratory, Department of Chemistry, Faculty of Exact Sciences, University of Mentouri Brothers, Constantine-1, 25000, Algeria.
[2]Biochemistry and Bacteriology Services, BEN-BADIS-Hospitalo-University Center, Constantine, 25000, Algeria.
[3]Laboratory of Natural Products of Plant Origin and Organic Synthesis, Department of Chemistry, University of Mentouri Brothers, Constantine-1, 25000, Algeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author NB designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. She performed the biological assay. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was focused on evaluation of antibacterial activity of 4,4'-diacetyldiphenylselenide against some bacterial species.
Introduction: Many compounds containing selenium element, notably 4,4'-diacetyldiphenylselenide, have started getting a great pharmacological interest as bioactive molecules insofar as the -*In vitro* - antimicrobial tests were carried out on different bacterial strains.

**Corresponding author: E-mail: nadjahorg@gmail.com;*

Methodology: The 4,4'-diacetyldiphenylselenide was synthesized in our laboratory and reported for the first time in 2010. The studied bacterial strains were collected from different places, some ones are standard strains and the others were clinically isolated and identified by biochemical tests.
The antibacterial property was evaluated via determination of Minimum Inhibitory Concentrations (MIC) using dilution method in the same conditions.
Results and Discussion: The most effective MICs are achieved at 16 µg/ mL for *Providencia rettgeri,* 32 µg/ mL for *Salmonella typhimurium*, 88 ±11.31 µg/ mL for *Serratia liquefaciens*, 128 µg/ mL for *Shigella dysenteriae,* 144 µg/ mL for *Morganella morganii,* 200 µg/ mL , 200 ± 11.31 µg/ mL for *Proteus mirabilis* and *Acinetobacter baumannii* respectively.
Conclusion: This study showed that 4,4'-diacetyldiphenylselenide is a bacteriostatic agent at determined Minimum Inhibitory Concentrations (MIC) against several pathogenic bacteria.

Keywords: Antimicrobial; bacteriostatic; In vitro; MIC; pathogenic.

1. INTRODUCTION

The organoselenium compounds are considered the key intermediates for the synthesis of pharmaceutical products [1-5], they have been reported to possess various biological activities, they mainly deal as anti-inflammatory, anti-oxidant [6-12], anti-allergic [13-16] and anticancer agents [17-18].

We have proceeded to study the influence of the organoselenide compounds on the microbial growth, in terms of evaluation of the antimicrobial activity of these products against some species that are most commonly responsible for various infections and intoxications, probably fatal if the organism is not provided with some basic antibiotics.

The aim of the present work was rigorously led to test the antibacterial effects, introduced by our product on different bacterial strains in order to promote its medicinal importance.

2. MATERIALS AND METHODS

The 4,4'-diacetyldiphenylselenide was previously prepared and identified in our laboratory using physicochemical analyses. The following figure presents the chemical structure of 4, 4'-diacetyldiphenylselenide (Fig. 1).

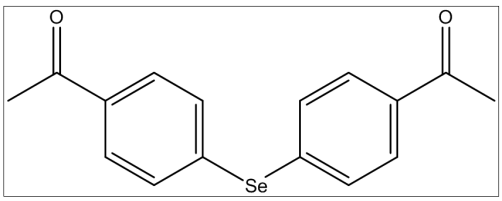

Fig. 1. 4,4'-diacetyldiphenylselenide structure

This product was practically checked in its antibacterial activity by (MICs) determination.

2.1 Technical Profile

Beside *diffusion method* which is generally used for applying Antimicrobial Disk Susceptibility Tests, the *dilution method* is gotten very useful by laboratories for clinical diagnosis:

2.1.1 Dilution method definition

This method is based on either broth or agar dilutions, for measuring quantitatively the *In vitro* activity of an antimicrobial agent against a given bacterial isolate according to the *Clinical and Laboratory Standards Institute (CLSI)* [19], the performance of the corresponding tests lies in preparation of a series of tubes or plates with a broth or agar medium to which various concentrations of the antimicrobial agents are added. The tubes or plates are then inoculated with a standardized suspension of the tested organism. After incubation at 35 ± 2°C, the tests are examined by determining the MIC which presents the lowest concentration of antimicrobial agent that inhibits the visible growth of microorganisms in agar or broth dilution susceptibility test.

2.1.2 General procedure

2.1.2.1 Preparation and dilution of inoculum suspension

The standardized inoculum must be prepared for agar dilution method by growing microorganisms and adjusting the microbial cultures to the 0.5 McFarland standard which contains about 1 to 2 × 10^8 colony forming units (CFU)/mL with most

species. The final inoculum on the agar will contain approximately 10 CFU per spot.

2.1.2.2 Inoculation of agar dilution plates

The prepared agar plates containing the different antimicrobial concentrations must be inoculated with standardized inoculums starting with the lowest concentration. It is advisable to inoculate a growth control plate without antimicrobial agent in the first and the last of inoculation to ensure there was no contamination or significant antimicrobial carryover during the inoculation.

2.1.2.3 Incubation of agar dilution plates

After drying the inoculated plates at room temperature, they must be subjected to incubation into oven at 35 ± 2°C for 16 to 20 hours. In the case of fastidious organisms, the oven must be equipped with CO_2 atmosphere.

2.1.2.4 Determination of MIC or agar dilution end point

The determination of *agar dilution end point* of microbial agent in a dark place must be set at the concentration in which there is 80% or greater reduction in growth as compared to the control, then the determination of MIC must be set at the lowest concentration of antimicrobial agent that completely inhibits microbial growth, disregarding a faint haze caused by the inoculums.

2.2 Antibacterial Activity Study

2.2.1 Preparation of product

2.2.1.1 Qualitative assay

A preliminary test suggested that the 4,4'-diacetyldiphenylselenide is absolutely soluble in ethanol (96%). Furthermore, the toxicity of ethanol was tested against the bacterial strains via disk diffusion method. In addition, a following control without solvent was also performed in order to prove that the solvent activity is almost negligible against studied bacteria.

2.2.1.2 Quantitative assay

A serial dilutions of the 4,4'-diacetyldiphenylselenide were made to obtain the concentrations ranged from 16 µg/mL to 800 µg/mL. The principal solution was prepared from dissolving 120 mg of this product in 15 mL ethanol (96%).

2.2.2 Preparation of tested bacteria

We were interested in performance of antibacterial activity of 4,4'-diacetyldiphenylselenide against in total 11 species (Gram positive and Gram negative bacteria). According to the book of performance standards for antimicrobial susceptibility testing, the examination of bacterial resistance showed that:

Staphylococcus aureus has homogenic resistance to β-lactams associated to aminoglycoside resistance.
Esherichia coli has a resistance to aminopenicilin, carboxypenicillin, ureidopenicillin.
Serratia liquefaciens has a resistance to aminopenicillins (ampicillin, amoxicillin+clavulanic acid), cephalosporin 1st generation (cefazolin).
Pseudomonas aeruginosa has a resistance to pefloxacin.
Klebsiella pneumoniae has a resistance to clavulanic acid associated to amoxicillin.
Proteus mirabilis has a resistance to trimethoprime- sulfamethoxazole, nalidixic acid, ticarcillin, amoxicillin and pefloxacin.

Table 1. The studied bacterial strains and their sources

The bacterial strain	The source
Escherichia coli ATCC 25922	*Pasteur Institute – Algiers*
Pseudomonas aeruginosa ATCC 27853	*Pasteur Institute – Algiers*
Proteus mirabilis	*Adenophlegmon sample*
Staphylococcus aureus ATCC 25923	*Pasteur Institute – Algiers*
Klebsiella pneumoneae	*Protected tracheal sample*
Salmonella typhimurium	*Urine sample*
Shigella dysenteriae	*Urine sample*
Serratia liquefaciens	*Tracheal sample*
Providencia rettgeri	*Pleural sample*
Morganella morganii	*Pus sample*
Acinetobacter baumannii	*Pus sample*

2.2.3 Preparation of antibacterial media

Cultural media of different strains were prepared in stationary phase by pricking about 0.3 mL of bacterial culture in tubes containing 10 mL *Mueller-Hinton* broth and they were incubated at 37°C for 18 hours.

The antibacterial activity was then realized using the dilution method following *the Clinical and Laboratory Standards Institute (CLSI) guidelines*.

18 mL of sterile *Mueller Hinton* (MH) agar (18 mL) were added to each *Petri* plate including the product dilution (2 mL) previously prepared from the low to the high concentration and then mixed carefully until the setting of agar. A control plate containing 2 mL of ethanol (96%) was added.

Afterward, *Petri* plates were inoculated with the microorganism inoculums (prepared with an overnight culture of tested microorganism and adjusted to 0.5 McFarland standard turbidity).

The plates were left at room temperature for 30 minutes and incubated at 37°C for 24 hours. Each test was done in duplicate through two different experiments.

3. RESULTS AND DISCUSSION

Our product 4,4'-diacetyldiphenylselenide showed an efficient antimicrobial activity against the different tested bacteria at different MIC values.

The ethanol used in several therapeutic preparations, solubilizes the active components of the organoselenide compounds.

In our dilution method, the bacterial species were tested for their ability to result in a visible growth on agar plates containing a series of our product dilutions. This procedure leads to determine the MIC values that are summarized in the following table (Table 2).

The agar dilution method, described in the report of international collaborative study of antimicrobial susceptibility testing [20], was attentively applied in order to evaluate the *In vitro*- antibacterial activity of our product against all strains by measuring the MICs insofar as the inhibitory ability of this organoselenide compound depends on its qualitative effect.

It is important to note that the inhibitory ability varies according to the studied species upon the concentration incidence.

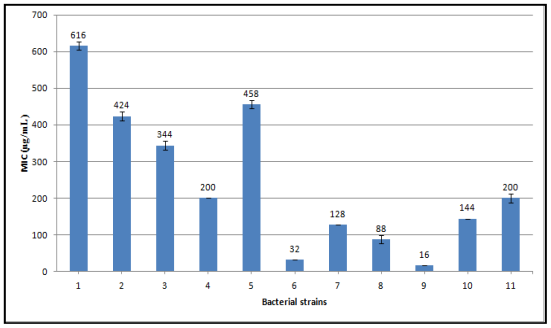

Fig. 2. The average MICs of 4,4'-diacetyldiphenylselenide for each tested bacterial strain

1: Staphylococcus aureus ATCC 25923
2: Escherichia coli ATCC 25922
3: Pseudomonas aeruginosa ATCC 27853
4: Proteus mirabilis
5: Klebsiella pneumoneae
6: Salmonella typhimurium
7: Shigella dysenteriae
8: Serratia liquefaciens
9: Providencia rettgeri
10: Morganella morgannii
11: Acinetobacter baumannii

Generally, the MICs determined in the same conditions, were found ranged from 16 µg/ mL to 624 µg/ mL. The values of the most effective concentrations are 16 µg/ mL for *Providencia rettgeri,* 32 µg/ mL for *Salmonella typhimurium*, 88 ±11.31 µg/ mL for *Serratia liquefaciens*, 128 µg/ mL for *Shigella dysenteriae,* 144 µg/ mL for *Morganella morganii,* 200 µg/ mL, 200 ± 11.31 µg/ mL for *Proteus mirabilis and Acinetobacter baumannii* respectively.

Table 2. The average MIC values of different bacteria

Bacterial strain	MIC_1 (µg/mL)	MIC_2 (µg/mL)	Average MIC
S. aureus ATCC 25923	624	608	616±11,31
E. coli ATCC 25922	416	432	424±11,31
P. aeruginosa ATCC 27853	336	352	344±11,31
Proteus mirabilis	200	200	200
Klebsiella pneumoneae	464	448	456±11,31
Salmonella typhimurium	32	32	32
Shigella dysenteriae	128	128	128
Serratia liquefaciens	96	80	88±11,31
Providencia rettgeri	16	16	16
Morganella morganii	144	144	144
Acinetobacter baumannii	192	208	200±11,31

All Gram-negative bacteria were inhibited using concentrations limited between 16 µg/ mL as a low concentration and 464 µg/ mL as a high concentration. Whereas Staphylococcus aureus ATCC 25923 which is a Gram-positive bacterium, a resistant strain to β-lactamin associated to aminosid, was found sensitive at 616 ±11.31 µg/ mL.

The comparison between the MICs and the resistance of the tested bacteria to the corresponding antibiotics reveals the influence of Gram-positive and Gram negative variation of bacteria. This strategy leads to deduce a relation between susceptibility of bacteria to 4,4'-diacetyldiphenylselenide and their resistance to antibiotics.

4. CONCLUSION

The study of the inhibitory ability "*In vitro*", using the dilution method showed that the 4,4'-diacetyldiphenylselenide has a bacteriostatic effect at determined MICs depending on bacterial species.

Taking into account the screening protocol of the standard strains including Gram positive and Gram negative bacteria, the product should be tested against these standard strains as microorganisms related to important infections. It has been reported that our product has an efficient antibacterial activity on Gram- negative bacteria more than Gram- positive bacteria. Indeed this methodical study in the presence of witness media showed that 4,4'-diacetyldiphenylselenide causes a complete growth inhibition at different MICs:

> 16 µg/ mL for *Providencia. rettgeri,* 32 µg/ mL for *Salmonella typhimurium,* 88 ±11.31 µg/ mL for *Serratia liquefaciens*, 128 µg/ mL for *Shigella dysenteriae,* 144 µg/ mL for *Morganella morganii,* 200 µg/ mL, 200 ±11.31 µg/ mL for *Proteus mirabilis* and *Acinetobacter baumannii* respectively.

The antimicrobial activity of organoselenide compounds is mainly related to their chemical composition, more particularly to the functional groups attached to their principal molecules (alcohols, aldehydes and phenols); in our case, the sites which mark an important activity are reported to the ketone group, and to the selenium element (more unstable than sulphur) which represents an oligo-element indispensable to the human and animal health, by its pharmaceutical properties, although it is toxic in high doses.

Moreover, it comes out from our work that the organoselenide compounds could have important therapeutic benefits. In fact, if these compounds are synthesized under severe conditions, other further studies are needed to elucidate whether the cell structure and permeability to these organoselenide molecules or even specific targets in the cell enzymatic systems are involved in microbial sensitivity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kondo S. Pharmacological studies on aromatic selenium compounds, Japanese Journal of Medical Sciences [Part] 4: Pharmacology. 1935;9:29-58.
2. Woods JA, Hadfield JA, McGown AT, Fox BW. Bioactivity and molecular modelling of diphenylsulfides and diphenylselenides. Bioorganic and Medicinal Chemistry. 1993; 1(5):333-340.
3. (a) Agenas LB, Gunther WH, Kayman DL, Organic selenium compounds: Their chemistry and biology. John Wiley and Sons: London; 1973.

b) Baird CP, Rayner CM, J. Chem Soc, Perkin Trans I. 1998;1973–2003.
4. Parnham M J, Erich Graf. Pharmacology of synthetic organic selenium compounds. Progress in Drug Research. 1991;36:9-47.
5. Hu Chun, Zhang Pu, Li Huiyuan, Ji Zhizhong, Liu Baili. Advances in organoselenium compounds used as pharmaceuticals. Huaxue Tongbao. 2002; 65(3):162-166,161.
6. Andersson CM, Hallberg A, Linden M, Brattsand R, Moldeus P, Cotgreave I. Antioxidant activity of some diarylselenides in biological systems. Free Radical Biology & Med. 1994;16(1):17-28.
7. Renson M, Dereu N. Benzisoselena-zolinones and derivatives, a new series of antiinflammatory and antioxidant agents. Journal de Pharmacie de Belgique. 1990;45(5):322–330.
8. Engman L, Stern D, Pelcman M, Andersson CM. Thiol peroxidase-activity of diorganyl tellurides. J. Org. Chem. 1994;59:1973–1979.
9. Anderson CM, Hallberg A, Hogberg T. Advances in the development of pharmaceutical antioxidants. Adv. Drug Res. 1996;(28):65-180.
10. Malmström J, Jonsson M, Cotgreave IA, Hammarström L, Sjödin M, Engman L. The Antioxidant Profile of 2,3-Dihydrobenzo[b]furan-5-ol and Its 1-Thio, 1- Seleno, and 1-Telluro Analogues. J. Am. Chem. Soc. 2001;123:3434–3440.
11. Mugesh G, Singh HB. Synthetic organoselenium compounds as antioxidants: Glutathione peroxidase activity. Chem. Soc. Rev. 2000;29:347-357.
12. Mugesh G, Du Mont WW, Sies H. Chemistry of biologically important synthetic organoselenium compounds. Chem. Rev. 2001;101:2125–2179.
13. Martinez-Ramos F, Salgado-Zamora H, Campos-Aldrete ME, Melendez-Camargo E, Marquez-Flores Y, Soriano-Garcia M. Synthesis and anti-inflammatory activity evaluation of unsymmetrical selenides. European Journal of Medicinal Chemistry. 2008;43(7):1432-1437.
14. Shamberger RJ, Biochemistry of selenium. Frieden E Ed. Plenum Press. 1983;7.
15. Abdel-Hafez Sh H. Selenium containing heterocycles: Synthesis, antiinflammatory, analgesic and antimicrobial activities of some new 4-cyanopyridazine- 3(2H)-selenone derivatives. European Journal of Medicinal Chemistry. 2008;3(9):1971-1977.
16. Galet V, Bernier JL, Henichart JP, Lesieur D, Abadie C, Rochette L, Lindenbaum A, Chalas J, De la Faverie J FR. Benzoselenazolinone derivatives designed to be glutathione peroxidase mimetics feature inhibition of cyclooxygenase/5-lipoxygenase pathways and anti-inflammatory activity. J. Med. Chem. 1994; 37(18):2903–2911.
17. Zongjian Z, Weiqin J. Selenium in prevention of cancer: Evidence and mechanism. Biomedical Research on Trace Elements. 2008;19(4):282-289.
18. Clement Ip, Lisk DJ, Ganther HE, Thompson HJ. Anticancer Res. 1997; 17(5A):3195-3199.
19. (CLSI) Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests f or bacteria that grow aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA. 2012;32(2).
20. Ericsson H, Sherris JC. Antibiotic sensitivity testing, Report of an international collaborative study. Acta Pathol Microbiol Scand Sect B. 1971; 217:Suppl 217:1.

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