2-*benzhydrylsulfinyl-N-hydroxyacetamide* extracted from fig: A good therapeutic agent against *Staphylococcus aureus*

Cite as: AIP Conference Proceedings **2213**, 020223 (2020); https://doi.org/10.1063/5.0000165
Published Online: 25 March 2020

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2-benzhydrylsulfinyl-N-hydroxyacetamide Extracted from Fig: A good Therapeutic Agent against *Staphylococcus Aureus*

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Abstract. *2-benzhydrylsulfinyl-N-hydroxyacetamide* was extracted from fig fruit. The fig fruits were placed in selective sequentially extract processes using soxhlet. The antibacterial activity of *2-benzhydrylsulfinyl-n-hydroxyacetamide* was tested against human pathogenic *Staphylococcus aureus* using the Optical density assy technique to measure bacterial growth curve. The alternation in the cluster shap of treated *S. aureus* was measured using SEM technique. The mechanism by which *2-benzhydrylsulfinyl-N-hydroxyacetamide* destroy bacterial strain was measured by investigation of reactive oxygen species (ROS) using Acridine orange-ethidium bromide (AO/EtBr) double staining assay. The results of the present study demonstrated that the 2-*benzhydrylsulfinyl-N-hydroxyacetamide* as a new DNA-mediated antimicrobial agent. *2-benzhydrylsulfinyl-N-hydroxyacetamide* was experimental new agent to breakdown the bacterial cells by permeating the bacterial genetic materials (nucleic acid and cytoplasmic membrane, leading to loss of cell-wall integrity, damage of nucleic acid, and increased in the permeability of bacterial cell-wall. The *2-benzhydrylsulfinyl-N-hydroxyacetamide* could serve as a novel active antimicrobial agent in biomedical applications.

INTRODUCTION

Plants, fruits and medicinal herbs are the main sources of most chemical drugs. The extraction and characterization of many active organic compounds derived from mature fruits have led to the generation of some highly effective drugs. It is recommended to know the chemical components of plant extracts and fruits because this information will be important for the installation of complex chemicals. Many studies has been reported that the plants contain several organic compounds with biological activities. Medicinal plant extracts also play a very necessary and essential roles in healthy case of human and may be of nutritional importance [1]. It is believed that raw extracts of some fruits and traditional herbes medicinal plants are biologically more active than synthetic chemical materials manufactured due to their synergistic effects. The chemical discovery of plants indicates the presence of many organic chemicals including alkaline flavonoids, tannins , steroids, glycosides, saponins and other medical organic compounds. Secondary metabolites of plants and fruits act as challenge mechanisms against predation by many cancer tumors and viral diseases of microorganisms [2]. The mature black figs and their extracts showed beneficial therapeutic effects, including antimicrobial, antioxidants and immunosuppressive effects. Because of the abundance of mature fig fruits leads to the abundance of organic compounds that play an important role in the prevention and treatment of diseases and can prevent or limit the negative effects of tumors and infections that infect tissue cells [3]. The fruits of mature figs can be an important source of chemical compounds of biological and pharmaceutical importance. This study was designed to extract *2-benzhydrylsulfinyl-N-hydroxyacetamide* from fig fruit and study it's biological activity against *S. aureus*. *2-benzhydrylsulfinyl-N-hydroxyacetamide* exhited potential activity against tested bacterial strain. Further works are needed to investigate the role of *2-benzhydrylsulfinyl-N-hydroxyacetamide* as an approach for managing other bacterial species other than the ones reported in this study.

2nd International Conference on Materials Engineering & Science (IConMEAS 2019)
AIP Conf. Proc. 2213, 020223-1–020223-5; https://doi.org/10.1063/5.0000165
Published by AIP Publishing. 978-0-7354-1964-3/$30.00

Taken together, the results of the present work demonstrated that *2-benzhydrylsulfinyl-N-hydroxyacetamide* could be considered as an effective and alternative treatment for prevention of microbial infection especially bacterial infections.

MATERIAL AND METHOD

Collecting Fig Fruit and Extraction

The fresh and dried figs were obtained from Iraq, on August, 2018. A sample of the plant was identified by a plant coordinator and the herb of the Center of Agricultural Research and Natural Sciences Applied, University of Basra. The collection and the weighted process were completed of 5 kg of figs with perfect wash by the DW in order to dispose of dust and impurities then the fruits were sliced into small pieces up to 1 gram per piece. The fresh and dried samples were subjected to selective sequential extraction by using solvents of increasing polarity, Therefore extraction solvents were evaporated using rotary dryer (Heidolph Rota vapour-Germany). *2-benzhydrylsulfinyl)-N-hydroxyacetamide* is diagnosed using of UV-Viss data not shown.

Antibacterial Activity of *2-benzhydrylsulfinyl)-N-hydroxyacetamide*

to investigate the effect of 2-benzhydrylsulfinyl)-N-hydroxyacetamide at the *S. aureus* viability and replication, the bacterial strains had been inoculated at 37°C on LB agar plates, and cultered had been accrued from the freshly colony plates into a new tubes containing 100 mL of brain heart infusion media. The samples of inoculated bacterial species were allowed to develop until the optical density (OD) of the brain heart infusion reached 0.1at 600nm, which corresponds a bacterial attention of 10^7 (CFU/mL) [4,5]. Then, 0.1mL of the bacterial cultures have been introduced to 5mL of sparkling nutrient broth include 2-benzhydrylsulfinyl)-N-hydroxyacetamide at the concentration 25µg/mL and incubated at 37°C for 24h. The growth of S. aureus was evaluated by reading the OD of the brain heart infusion every 6-hrs [6,7].

A study of *S. Aureus* Morphology Using SEM

A scanning electron microscope (SEM) technique was used to observe the changes in morphology *S.aureus*. First of all, the *2-benzhydrylsulfinyl)-N-hydroxyacetamide* treated and non-treated bacterial cells with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* at concentration 25 µg/ml were centrifuged at 3000 rpm for 20 mimutes, then the sampled washed five times with sterial PBS [8,9]. A small drop of bacterial suspension was added on glass slide. The slides were left to dried at room temperature before being fixation. The fixed slides were incubated at 37ºC for three hr, then, dehydrated in an ascending grade of methanol, air-dried, mounted on the SEM stubs, and coated with for 5 min with GNPs, leaving a 50 nm of GNPs on the surface of the cells. The samples examined under an SEM (TESCAN, Vega III, Czech Republic) [10,11].

Release of Reactive Oxygen Species

Acrydin orange (AO)/ Ethidium bromide (EtBr) staining assay was used to investigate the release and activity of ROS as described by [12,13,14,15].

Statistical Analysis

The statistical analysis was done depending on the unpaired t test. p value of < 0.05 was considered signification [16,17].

RESULT AND DISCUSSION

Antibacterial Activity of 2-benzhydrylsulfinyl)-N-hydroxyacetamide

The antibacterial activity of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* was studied using *S.aureus*. The *2-benzhydrylsulfinyl)-N-hydroxyacetamide* showed a time-dependent effect on the growth of the studied organisms, especially after 6-24 h of treatment (Figure 1, 2) respectively.

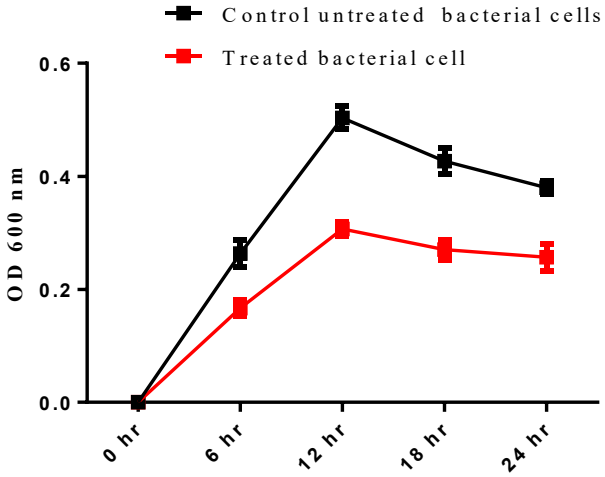

FIGURE 1. Effect of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig in growth curve rate of *S.aurues*. The data are shown as the mean±SEM.

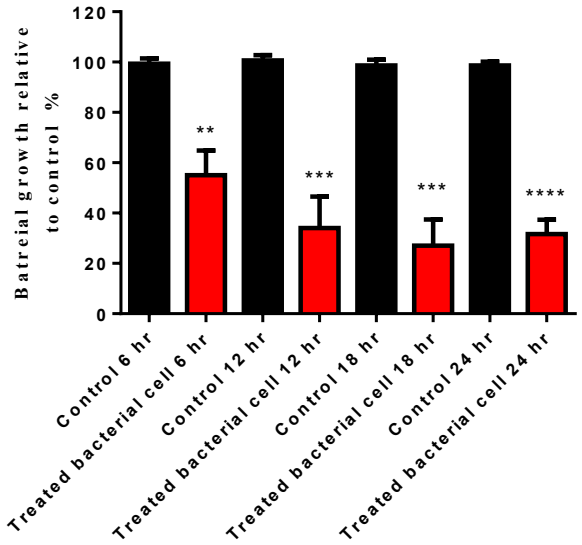

FIGURE 2. Bacterial growth of *S.aurues* relative to control after treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig. The value results are presented as the mean ±SEM. **p<0.01, ***p<0.001, ****p<0.0001.

Effect of 2-benzhydrylsulfinyl)-N-hydroxyacetamide in S.aureus Morphology

The effect of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* which is extracted from fig on the shape and size of the *2-benzhydrylsulfinyl)-N-hydroxyacetamide* treated *S.aureus* strains was studied using an SEM. The obtained SEM images showed the differences in the bacterial cells structures befor and after treated with *2-benzhydrylsulfinyl)-N-*

hydroxyacetamide. *S.aureus* is commonly exist in clusters; they were observed to exist a singly and in pairs after treatment with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* (Figure 3). The SEM images shows bacterial membrane rupture after treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide*.

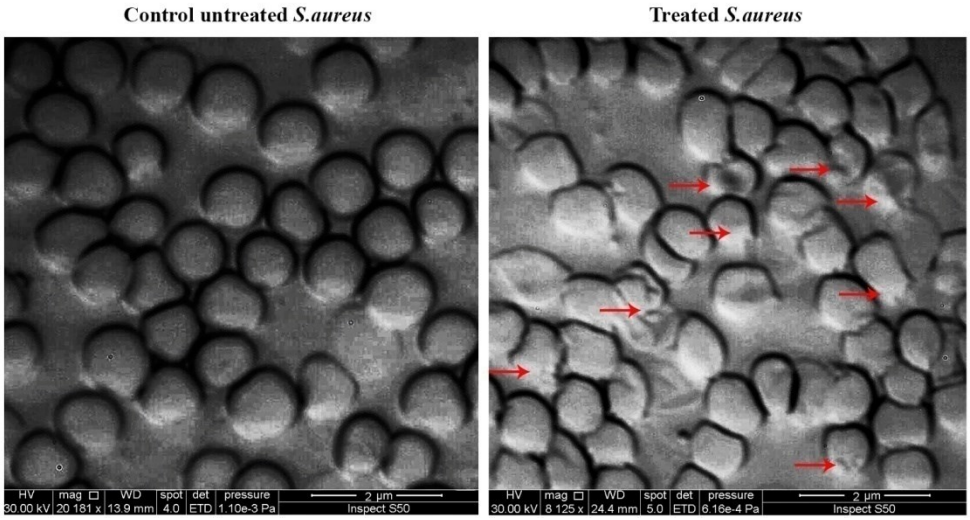

FIGURE 3. SEM images of *S.aureus* treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig, treated bacterial cells showing membrane damage as indicated .

2-benzhydrylsulfinyl)-N-hydroxyacetamide Enhances Production of ROS

The production of ROS by *S.aureus* after treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig was done using an Acrydin Orange AO/ Ethidium bromide EtBr staining assay. This assay detects most of free radicals such as hydrogen peroxide and nitric oxide as ROS formation and activity indicators. The AO/EtBr dye, upon contact with the ROS produced by the stressed organisms, gets oxidized. The components of Ethidium bromide (EtBr) stain could permeates only the cells that have impaired and disfuncion in membrane integrity and interact with the nucleic acid of the cells. The viable bacterial cells are stained green while the non-viable bacterial cells with nuclear damage are stained red [18,19]. While the bacterial cells which is non treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* appeared in normal structure and green in colour, while treated *S.aureus* with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig shows a highly structural and morphological changes, deformities and high amount and levels of ROS activity and production (Figure 4). We done this test to measuring the ability of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* to induce death of tested *S.aureus*, a combination of acridine orange-ethidium bromide dyes was used (Figure. 4). In this assay, the structure of the nuclei of the was observed to be intact, with a stable bright green color. The membranes of bacterial cells treated with *2-benzhydrylsulfinyl)-N-hydroxyacetamide* less integrity in comparison to the membranes of the untreated bacterial cells. Apoptotic cells commonly show nuclei that are characterized by red to green color while the condensation of their chromatin varies in level. The morphological alterations in the treated bacterial cells suggested that the observed cell death was caused by apoptosis rather than necrosis. The apoptotic bacterial cells were evaluated based on DNA damage. In this study, the efficiency of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* which is extracted from fig at concentration 25µg/mL, was studied. AO-EB dual staining was used to distinct apoptotic signs characteristics to nucleate alternations. Viable bacterial cells were shown green and apoptotic cells were shown orange or red as they stained with EtBr figure-4. Exposure of cells to *2-benzhydrylsulfinyl)-N-hydroxyacetamide* caused an increase in membrane disruptionand formation of lysosomes vacuoles compared to control untreated bacterial cells. The results of this study demonstrated that the high ability of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* to cause death to the bacterial cell is due to the ability of these materials to penetrate through the cell membrane and effect on the genetic materials.

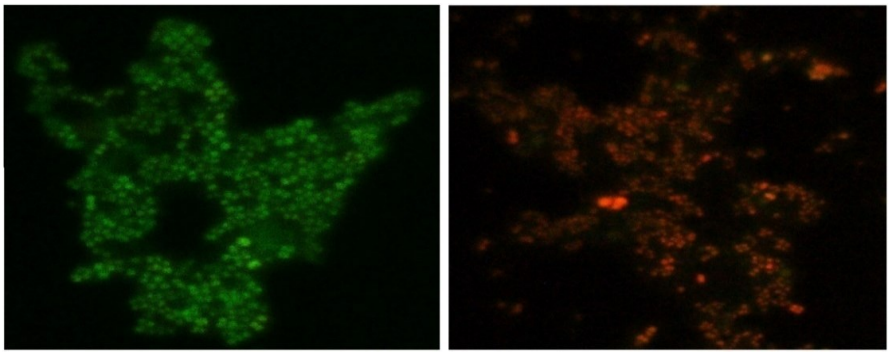

FIGURE 4. Images of the stained *S.aureus* bacterial strains. Green fluourecent stain indicated a live strains. While, red one indicated died bacterial strains in absence and presence of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* extracted from fig.

CONCLUSIONS

In conclusion, the present work was designed to measure the antimicrobial and antibacterial activity of *2-benzhydrylsulfinyl)-N-hydroxyacetamide*. We have demonstrated that the *2-benzhydrylsulfinyl)-N-hydroxyacetamide* showed inhibitory activity against *S.aureus*. Taken together, they obtained results exhibited a potential antibacterial activity of *2-benzhydrylsulfinyl)-N-hydroxyacetamide* against *S. aureus*. These results confirm that the *2-benzhydrylsulfinyl)-N-hydroxyacetamide* could be one of key factor determining the anti-inflammatory activity during acute infection. It could be serve as a promising, new therapeutic model in future. Taken together, the results of the current work proved that *2-benzhydrylsulfinyl-N-hydroxyacetamide* could be considered as an effective treatment model for prevention of microbial infection especially bacterial infections.

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