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Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol

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ABSTRACT

The indiscriminate use of antimicrobial drugs has increased the spectrum of exposure of these organisms. In our studies, these phenolic compounds were evaluated: gallic acid, caffeic acid and pyrogallol. The antibacterial, antifungal and modulatory of antibiotic activities of these compounds were assayed using microdilution method of Minimum Inhibitory Concentration (MIC) to bacteria and Minimum Fungicide Concentration (MFC) to fungi. The modulation was made by comparisons of the MIC and MFC of the compounds alone and combined with drugs against bacteria and fungi respectively, using a sub-inhibitory concentration of 128 μg/mL of substances (MIC/8). All substances not demonstrated clinically relevant antibacterial activity with a MIC above ≥1024 μg/mL. As a result, we observed that the caffeic acid presented a potentiating antibacterial effect over the 3 groups of bacteria studied. Pyrogallol showed a synergistic effect with two of the antibiotics tested, but only against *Staphylococcus aureus*. In general, caffeic acid was the substance that presented with the greatest number of antibiotics and with the greatest number of bacteria. In relation to the antifungal activity of all the compounds, the verified results were ≥1024 μg/mL, not demonstrating significant activity. Regarding potentiation of the effect of fluconazole, was observed synergistic effect only when assayed against *Candida tropicalis*, with all substances. Therefore, as can be seen, the compounds presented as substances that can be promising potentiating agents of antimicrobial drugs, even though they do not have direct antibacterial and antifungal action.

1. Introduction

Bacteria are microorganisms inseparable from life on Earth, which can be found anywhere such as: lining the skin, the mucosa and gastrointestinal tract. Some bacteria and fungi are harmless, acting in a beneficial way to its host and providing nutrients or protection against pathogens and diseases. The indiscriminate use of antibiotics increases the spectrum of exposure of microorganisms to these, in addition to initiating an increase in selection pressure, facilitating the development of resistance mechanisms to these microorganisms [1].

Antimicrobial resistance has become the main problem in public health in the world, which affects all countries [2]. In Europe and North America, it is already known that *Staphylococcus aureus* is methicillin resistant (MRSA), *Streptococcus pneumoniae* is penicillin resistant (PNSSP), enterococci are resistant to vancomycin (VRE) and Enterobacteriaceae are extended-spectrum beta-lactamase (ESBL) producers that have disseminated [3].

The Gram-positive *Staphylococcus aureus* bacteria represents the most common etiological agent for purulent infections, for example, furuncle, carbuncle, abscess, myocarditis, endocarditis, pneumonia, meningitis, bacterial arthritis [4]. Whereas the Gram-negative

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Pseudomonas aeruginosa bacteria is responsible for a variety of infections, such as those affecting the skin, urinary tract, ears and eyes, they also possess numerous structural factors, enzymes and toxins that potentiate their virulence, in addition to making them resistant to the most common antibiotics [5]. *Escherichia coli*, another Gram-negative bacteria, is one of the main causes of human infectious diseases. It is known for producing enterotoxins whose properties and participation in diarrhea have been widely investigated [6,7], especially in urinary tract infections [8].

Microbial infections are not only related to bacteria, opportunistic fungi are also responsible for infections, especially those caused by the *Candida* genus. This is one of the most highlighted as causing superficial and invasive infections, ranging from commensals to important infectious agents of the organism, normally found in the vagina and gastrointestinal tract [9]. According to Morais-Braga et al. [10], *Candida* spp. is an opportunistic pathogen which can compromise any part of the human body, causing candidiasis.

Candidiasis is the most commonly caused mycosis by opportunistic fungi. The main species associated with this disease are *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata* and *Candida krusei* [11]. These yeast are present in the skin and mucosal microbiota, become pathogenic in immunodeficient or immunosuppressed patients [12].

The clinical manifestations of candidiasis present great diversity in clinical scenarios, which can be divided in invasive or systemic candidiasis and mucocutaneous (oral, intertriginous, balanopreputial, vulvovaginal, paronychia and chronic mucocutaneous). Invasive candidiasis is a profound and serious infection that affects immunocompromised individuals. Any organ can be affected, thus, there is ocular, hepatosplenic, pulmonary, cardiac, central nervous system, musculoskeletal, myositis, arthritis, among other, candidiasis [13].

Phenolic or polyphenol compounds constitute one of the most numerous groups of plant secondary metabolites. It is estimated that more than 8000 structures are known. Natural phenols can be from simple molecules (benzoic and cinnamic acid) to highly polymerized components (lignin, melanin, tannin), with the flavonoids representing the most common and widely distributed subgroup [14]. Phenolic acids are characterized by the presence of a benzene ring, a carboxylic grouping and one or more hydroxyl and/or methoxyl groupings in the molecule, which confer antioxidant properties [14].

The metabolite gallic acid, for example, is derived from the shikimic acid, an intermediate of secondary metabolism, and is a component of hydrolysable tannins in plants [15]. Pyrogallol is a hydroxylated compound with proven antimicrobial action, such that its mechanism of action occurs through enzymatic inhibition by oxidized compounds [16]. The polyphenol caffeic acid is a compound found in many species that present proven antimicrobial activity, however the activities of this compound have not been analyzed in an isolated manner [17].

Thus, the work developed in the present study, shows unprecedented importance, especially in the case of the evaluation of antimicrobial modulation through the use of these phenolic compounds. Therefore, the objective of this work was to investigate the biological activities of the isolated caffeic acid, gallic acid and pyrogallol substances, using *in vitro* experimental models against multiresistant bacterial and fungal strains.

2. Materials and methods

2.1. Studied compounds

The phenolic compounds gallic acid, caffeic acid and pyrogallol, were evaluated, all of which were acquired from Sigma ChemicalCo. (St. Louis, MO, USA).

2.2. Microorganisms

The microorganisms used in the *in vitro* tests were obtained from clinical isolates (Table 1). The bacterial lineages used were *Escherichia coli* 06, *Staphylococcus aureus* 10 and *Pseudomonas aeruginosa* 15 strains. The fungi lineages used were: *Candida albicans* ATCC 40042 and *Candida tropicalis* ATCC 40006. All the fungal strains were maintained in Sabourad dextrose Agar (SDA,DifcoLaboratories Ltda.) and the bacterial strains in heart infusion (HIA, Difco Laboratories Ltda.). Before the assays, the lineages were cultured for 24 h at 37 °C in brain heart infusion broth (BHI, DifcoLaboratories Ltda.) for the bacteria, and the fungi in Sabourad dextrose broth (SDB, DifcoLaboratories Ltda.).

2.3. Drugs

To evaluate the antibacterial and antifungal activity as well as the modulatory antibiotic and antifungal actions of the compounds various drugs were used with Imipenem, Gentamicin and Norfloxacin as standard antibacterials, and Fluconazole was used as an antifungal. The drugs were obtained from the Sigma ChemicalCorp. Laboratory (St Louis, MO, USA). All the solutions were prepared based on the established recommendations in CLSI [18].

2.4. Minimum inhibitory concentration (MIC)

The MIC (minimum inhibitory concentration) of the phenolic compounds and of DMSO were determined in broth microdilution assays, using a 100 μL inoculum of each lineage, in a concentration of 10^5 CFU/mL, in BHI medium, in microdilution plates with 96 wells. In each well 100 μL of each sample solution will be added, followed by a 1:1 serial dilution, for both the three compounds in study as well as for the DMSO control. The final compound concentrations vary between 512 and 8 μg/mL. The plates were incubated at 37 °C for 24 h and, after this period, readings were evidenced by the use of Resazurin in the tests with bacteria and by medium turbidity in the fungal analysis, where the samples were analyzed in a spectrophotometer. The MICs were defined as the lowest concentrations necessary for the inhibition of growth. Specifically in the antifungal assays, the MFC was calculated by subcultivation of each well in a Petri dish with help from a wooden rod, later the plates were incubated for 24 h at 37 °C, and fungal growth was analyzed in a solid medium plate, to avoid problems in the observation due the flocculation of *Candida* cells [19,20].

2.5. Verification of antimicrobial activity alteration

For the evaluation of the phenolic compounds actions as microbial resistance modulators, the MIC was determined with antimicrobial and antifungal drugs, in the presence of the compounds, which were at sub-inhibitory concentrations (MIC/8) of 128 μg/mL, compared to the negative control DMSO and positive control containing microorganisms. These presented themselves in a concentration of 10^5 CFU/mL, suspended in BHI medium, in microdilution plates with 96 wells. Later, the addition of drugs proceeded a quantity of 100 μL distributed in each sterile microplate well, proceeding a 1:1 serial microdilution. The antibacterial and antifungal concentrations ranged between 2.44 and 2500 μg/mL and 8–512 μg/mL, respectively [21]. The plates were incubated at 37 °C for 24 h and following this period, readings were taken with the use of Resazurin in the modulation plates with antibiotics and by observation of turbidity in modulation plates with antifungals.