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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.07.016

Antibacterial activity of five Peruvian medicinal plants against *Pseudomonas aeruginosa*

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ARTICLE INFO

Article history:
Received 22 Jun 2015
Received in revised form 27 Jun, 2nd revised form 1 Jul, 3rd revised form 2 Jul 2015
Accepted 25 Jul 2015
Available online 18 Aug 2015

Keywords:
Pseudomonas
Medicinal plants
Maytenus macrocarpa
Dracontium loretense Krause
Tabebuia impetiginosa
Eucalyptus
Uncaria tomentosa

ABSTRACT

Objective: To evaluate the susceptibility of *Pseudomonas aeruginosa* (*P. aeruginosa*) *in vitro* to the ethanolic extracts obtained from five different Peruvian medicinal plants.
Methods: The plants were chopped and soaked in absolute ethanol (1:2, w/v). The antibacterial activity of compounds against *P. aeruginosa* was evaluated using the cup-plate agar diffusion method.
Results: The extracts from *Maytenus macrocarpa* ("Chuchuhuasi"), *Dracontium loretense* Krause ("Jergon Sacha"), *Tabebuia impetiginosa* ("Tahuari"), *Eucalyptus camaldulensis* Dehn (eucalyptus), *Uncaria tomentosa* ("Uña de gato") exhibited favorable antibacterial activity against *P. aeruginosa*. The inhibitory effect of the extracts on the strains of *P. aeruginosa* tested demonstrated that *Tabebuia impetiginosa* and *Maytenus macrocarpa* possess higher antibacterial activity.
Conclusions: The results of the present study scientifically validate the inhibitory capacity of the five medicinal plants attributed by their common use in folk medicine and contribute towards the development of new treatment options based on natural products.

1. Introduction

In 2014, the World Health Organization released its first report on surveillance of antimicrobial resistance, revealing that this is an increasing global threat and putting our capacity to treat common nosocomial or community-acquired infection at risk [1].

This growing problem was characterized by the infectious diseases caused by multidrug-resistant Gram-negative bacteria that challenge the public health policies worldwide at the point of being known as the ESKAPE pathogens [*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterobacter* spp.]. This term represents their escape from the effects of the antibacterial agents or the nonexistence of newer and more effective antibiotics [2].

P. aeruginosa is the most important toxigenic pathogen within the genus *Pseudomonas* because of the quantity and types of invasive infections it produces, as well as the noteworthy morbidity and mortality associated [3]. This Gram-negative bacterium has the ability to survive in adverse environments and develop multiple antibiotic resistance mechanisms. Among them, the most representative are the expression of chromosomal-encoded AmpC β-lactamase, the reduction of porin channels, the production of extended-spectrum β-lactamase and the mutation of topoisomerase II and IV [4]. It must be considered that several resistant mechanisms can coexist in one strain and just one of them can be effective against numerous antimicrobials [5].

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Peer review under responsibility of Hainan Medical University.

The resistant mechanisms in *P. aeruginosa* are related to enhancement of the mortality rate of patients infected with this pathogen [6]. Furthermore, this rate is higher among patients infected with resistant strains and received inappropriate initial empirical treatment [4]. Additionally, the rising indiscriminate use of antimicrobials in health centers or by people who practice self-medication could lead susceptible patients to get infected by multidrug-resistant microorganisms [7,8]. The emergence of antibiotic resistance and related toxicity issues limit the use of these drugs, and generate a renaissance in phytotherapy research [9]. To address this challenge, there is growing interest in identifying and evaluating antimicrobial compounds in extracts of medicinal plants as a new source of drugs and alternative treatment approach [10].

Of all the regions in the world with a diverse flora that can naturally provide medicinal plants, Peru is a privileged one. It possesses approximately 20 000 plant species, which is equivalent to 8% of the total number of plants in the world. Most of them are native plants or grow in the Peruvian Amazon. Nevertheless, probably less than 1% of the species have been studied to determine their phytochemicals with potential medicinal value [11,12].

The aim of present study was to evaluate the antibacterial capacity of five traditionally used Peruvian plants against *P. aeruginosa* in order to validate scientifically the inhibitory activity attributed by their popular use and to propose new sources of antimicrobial agents.

2. Materials and methods

2.1. Collection of plant materials

The plants *Maytenus macrocarpa* (*M. macrocarpa*) (common name: "Chuchuhuasi"), *Dracontium loretense* Krause (*D. loretense*) (common name: "Jergon Sacha"), *Tabebuia impetiginosa* (*T. impetiginosa*) (common name: "Tahuari"), *Eucalyptus camaldulensis* Dehn (*E. camaldulensis*) (common name: eucalyptus), *Uncaria tomentosa* (*U. tomentosa*) (common name: "Uña de gato") and *Allium sativum* (*A. sativum*) (common name: garlic) used in this study were purchased from naturist houses and six of them had sanitary registration.

2.2. Preparation of plant extracts

The plants were chopped and soaked in absolute ethanol (1:2, w/v) under shade for 10 days at room temperature. The mixtures were filtered through a Whatman No. 4 filter paper and the filtrates were evaporated at 50 °C [13]. All extracts were stored at 4 °C until use.

2.3. Bacterial test strain and growth conditions

For this study, a strain of *P. aeruginosa* (ATCC 55925) was used and provided by the Microbiology Laboratory of the Institute of Nutritional Research. The cultivation medium was trypticase soy agar (trypticase soy broth) (Oxoid, Hampshire, UK), supplemented with 10% defibrinated sheep blood. Cultures were grown aerobically for 24 h at 37 °C. For antibacterial activity assay, three or four isolated colonies were inoculated in 3 mL of brain heart infusion (BHI) broth and incubated without agitation for 24 h at 37 °C. The cultures were later diluted with fresh medium to approximate the density of 0.5 McFarland standard, which represented an estimated concentration of 1.5×10^8 CFU/mL.

The McFarland standard was prepared by inoculating colonies of the bacterial test strain in sterile saline and adjusting the cell density to the concentration specified before [14].

2.4. Antibacterial screening of the ethanolic extracts

2.4.1. Determination of antibacterial activity

To determine the antibacterial activity of studied extracts, the cup-plate agar diffusion method was used [15]. BHI agar was autoclaved for 15 min at 121 °C and cooled to about 40–42 °C. The medium was then inoculated with 0.1 mL of the prepared bacterial suspension, mixed gently and finally poured into sterile Petri dishes. These agar plates were incubated under sterile conditions for 8 h at room temperature. Three wells per plate of 6 mm in diameter and 4 mm in depth were made with the help of a sterile cork borer, preserving a distance of 3 cm between them. The wells were filled with 100 μL of the corresponding ethanolic extract. The extract from *A. sativum* (control of the extraction process) and ciprofloxacin (32 g/mL) (commercial control) were used as positive controls [8,16]. The Petri dishes were incubated under the same growth conditions as mentioned above. At the end of the period, the inhibition zones formed were measured in millimeters using the vernier. The inhibition zones less than 12 mm in diameter were not considered for the antibacterial activity analysis. For each extract, 12 replicates were assayed.

2.4.2. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum bacteriostatic concentration (MBSC)

The MIC was determined using the microdilution method as described by Jayaraman *et al.* [17] and Clinical and Laboratory Standards Institute [18]. Serial two-fold dilutions of all the extracts were prepared with sterile saline in a 96-well microtiter plate, obtaining a concentration range from 100 to 1.56 mg/mL. Then, 5 μL of *P. aeruginosa* suspension (optical density at 550 nm = 0.6) were added to the wells containing the dilutions. Each dose was assayed in quadruplicate. Uninoculated wells containing sterile saline or saline and extract were used as controls. After incubation for 24 h at 37 °C, the samples were observed. MIC was recorded as the lowest concentration of each plant extract that inhibited the bacterial growth as detected by the absence of visual turbidity.

To estimate the MBSC, an aliquot of each well that did not show microbial growth in the prior tests was swabbed on the entire surface of BHI agar plates and then incubated under the growth conditions described before. Subsequently, the lowest concentration of extract at which there was growth after subculturing was considered as the MBSC. In contrast, the lowest concentration that prevented the bacterial growth was registered as MBC.

2.5. Statistical analysis

All data were analyzed using SPSS package (version 21.0.0) for statistical analysis. For further inferential statistical analysis, Levene's and Welch's tests were carried out.