Antimicrobial Activity of Phenolic Compounds Extracted from *Platanus hybrid*a: Exploring Alternative Therapies for a Post-Antibiotic Era †

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Abstract: Multidrug-resistant bacteria are a significant threat to public health and new classes of antibiotics and approaches to treatment are needed. Several studies have shown that natural plant-derived compounds could be a promising mean to fight microbial resistance but only a few were conducted with antibiotic resistant bacteria. Therefore, the aim of this study was to extract phenolic compounds from the leaves, fruits, and tree trunk of *Platanus hybrid*a and evaluate their antimicrobial activity against antibiotic resistant bacterial strains. The polyphenolic compounds were extracted using a water/ethanol (20:80) mixture. Two grams of powder of each sample was extracted with 100 mL of solvent by stirring for 2h. The extracts were redissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL. An antimicrobial susceptibility assay was performed using the Kirby–Bauer disc diffusion method and was tested against ten different bacteria: *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The fruits had the highest antibacterial activity showing a minimum inhibitory concentration (MIC) of 10mg/mL, contrary to the tree trunk that showed the lowest antibacterial activity. None of the extracts showed antimicrobial properties against *S. enteritidis*, *E. faecium* and *E. faecalis*. These results show that *P. hybrid*a’s phenolic compounds act as antibacterial agents, which may become useful therapeutic tools and represent a source for the development of novel antimicrobials. However, they were not effective against all bacteria, which shows that polyphenols alone might not substitute antibiotics.

Keywords: polyphenols; plant-derived compounds; plane tree; antimicrobial activity; bacterial resistance; public health
1. Introduction

Antibiotics are substances with the capacity to selectively inhibit or kill microorganisms [1]. Their discovery was one of the greatest scientific breakthroughs of the 20th century and they became routinely used not only in human medicine but also in livestock production as therapeutic agents or growth promoters [2]. Misuse of antibiotics has resulted in the emergence of resistant bacteria against at least one antibiotic, which has generated a problem that affects public health. Bacteria have a remarkable capacity to adapt to adverse environmental conditions, which allows them to remain active even when in contact with antimicrobial substances [1,3]. Many research studies have shown a growing number of infections caused by resistant bacteria in hospitals and communities [4]. Bacterial resistance has accompanied the development and commercialization of antibiotics. At the beginning of the antibiotic era, concern was focused on resistance in Gram-positive bacteria. However, in recent decades, attention has shifted to the susceptibility pattern of Gram-negative bacteria [5]. Among the Gram-positives, *Staphylococcus aureus* stands out for causing infections ranging from simple skin diseases to severe conditions such as bacteremia and endocarditis. *Escherichia coli* has resistance to several drugs, such as carbapenemases and cephalosporins, among others. *Pseudomonas aeruginosa* are Gram-negative bacteria with high virulence. These are only some examples of bacteria that act as opportunistic microorganisms that can cause severe infections and even sepsis [6]. According to data obtained by the World Health Organization (WHO), the ability of bacteria to resist to the available antibiotics causes about 700 thousand death per year and it is estimated that this number will increase considerably, reaching 10 million deaths per year by 2050. In addition to this, antimicrobial resistance will have economic implications: studies have shown that the world may lose between 60 and 100 trillion dollars in economic production, which represents a decrease of 2.0 to 3.5% of global gross domestic product (GDP) expected for 2050 [3]. This scenario has led to the development of research aimed at identifying new antimicrobial agents through chemical synthesis or isolation from natural products [6].

Plants have been used for many centuries for therapeutic purposes. It is an ancient practice that has been empirically transmitted from generation to generation [6,7]. The WHO estimates that 80% of the population of some Asian and African countries presently uses medicinal plants to treat many diseases. Several scientific studies realized in the USA and Europe have revealed that their use has increased in recent years, which proves their important therapeutic effect [7]. The therapeutic properties of medicinal plants have been attributed to the presence of secondary metabolites that play a critical physiological role in these organisms but also interfere with pharmacological targets in humans and many other species [4]. A large number of plants have been screened as a source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds, which help the human body to reduce oxidative damage and provide protection against heart disease, cancer and Alzheimer’s disease. Furthermore, it has already been demonstrated that several plant-derived polyphenolic compounds exhibit important antimicrobial, antioxidant, anticancer and apoptosis-inducing properties [7]. *Platanus hybridra* Brot. (syn. *Platanus x acerifolia* (Ait.) Willd, *Platanus x hispanica* Mill. Ex Münchh), also known as London plane, is a hybrid between *Platanus occidentalis* L. (American origin) and *Platanus orientalis* L. (Oriental origin) [8,9]. This species belongs to the Platanaceae family and it was first reported in Europe in the 17th century [10]. These trees usually flower from March to April and fruit in late summer and autumn [8]. Since its appearance, *P. hybridra* has been valued highly as an ornamental tree providing shade and as a source of wood for different uses [9,10]. As a result, it is a very popular street tree in Europe and elsewhere in the world. In addition, it is widely used as an urban tree because it provides a large number of ecosystem services, grows fast, has a good tolerance to urban microclimate conditions and it is quite resistant to soil compaction and air pollution. The London plane tree’s pollution tolerance is due to its ability to accumulate pollutants in its cortex and most importantly because of its high capacity to capture particulate matter (PM) due to the morphological characteristics of its leaves [9]. Considering the current need to search for alternative sources of antimicrobial compounds or compounds with antibiotic resistance-modulatory effects, in this work, we extracted the phenolic compounds of the
leaves, fruits and trunk of this plane tree and investigated their antimicrobial activity against a wide range of multidrug-resistant bacteria.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

Plant material used included three plane tree components, namely the trunk, fruits, and leaves. Samples were collected in July 2020 in the North of Portugal. These components were manually separated, lyophilized, mill-powdered, and stored at −20 °C. The polyphenolic compounds were extracted using a water/ethanol (20:80) mixture. Two grams of powder of each powdered sample was extracted with 100 mL of solvent by stirring for 2 h. Samples were centrifuged for 15 min at 10,000 RPM. The supernatants of each extraction were collected, filtered, and the solvent evaporated on a rotary evaporator at 40 °C under reduced pressure. Finally, the obtained dry residues were weighted and redissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL for the analysis of antimicrobial activity. The extracts were stored under −20 °C until further analysis.

2.2. Antibacterial Activity

2.2.1. Bacterial Strains

Antimicrobial susceptibility testing was performed against six multidrug-resistant Gram-positive bacteria (Listeria monocytogenes, Bacillus cereus, Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis) and four multidrug resistant Gram-negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enteritidis and Escherichia coli). The strains are part of the University of Trás-os-Montes and Alto Douro and University of La Rioja collections. All the bacterial strains were subcultured from the original culture in brain heart infusion (BHI) agar (Oxoid, Basingstoke, UK) for 24 h at 37 °C. Muller–Hinton (MH) agar (Oxoid, Basingstoke, UK) was used for the antimicrobial susceptibility assay.

2.2.2. Antimicrobial Susceptibility Test

The antimicrobial susceptibility assay was performed using the Kirby–Bauer disc diffusion method. Each bacterial strain was seeded in BHI agar plates and incubated overnight at 37 °C. Colonies were suspended in physiological solution to a turbidity equivalent to 0.5 McFarland standard and inoculated on MH plates. The initial extract solution of 100 mg/mL was diluted with DMSO to 75, 50, 25, and 10 mg/mL and tested against the ten multidrug-resistant bacteria for the evaluation of antimicrobial susceptibility. Twenty microliters of each extract concentration was loaded on sterile blank discs (6 mm diameter) and the discs were placed on the inoculated MH plates, which were incubated for 24 h at 37 °C. The inhibition zones were measured with a ruler, recorded, and considered as indication for antibacterial activity.

3. Results and Discussion

The assessment of antimicrobial activity by P. hydrida’s components was performed using the Kirby–Bauer disc diffusion method. The results for the minimum inhibitory concentration (MIC) are expressed in Table 1. All extracts showed antimicrobial activity with clear-cut inhibition zone and as expected, different extracts exhibited different antimicrobial effects. Nevertheless, it is important to notice that, unlike most studies, we used antibiotic resistant bacteria, which have several mechanisms that confer them resistance to antibiotics and several natural compounds.

L. monocytogenes was the only bacteria that showed susceptibility to all the extracts. Ceruso et al. (2020), explored the potential antibacterial activity of an extraordinary vast collection of plant extracts against this pathogen and demonstrated similar results [11]. Phenolic compounds are frequently more effective against Gram-positive bacteria because the entry of these compounds into Gram-negative cytoplasm is hindered by the repulsion between lipopolysaccharide present on the surfaces
of Gram-negative bacteria and phenols [12, 13]. However, the phenolic compounds of our study did not have any effect against two Gram-positive bacteria (*E. faecium* and *E. faecalis*) and one Gram-negative bacteria (*S. enteritidis*). The susceptibility results against *S. enteritidis* are in agreement with the results obtained in other studies with phenolic extracts of winery by-products. The bacterial resistance to phenolic compounds is not fully understood but the same results of antimicrobial activity of *E. faecalis* and *E. faecium* could be explained by the fact that they share, not only the genus Enterococcus, but also similar antibiotic resistances and resistance genes [13].

Table 1. Minimum inhibitory concentration (MIC, mg/mL) and inhibition zones (mm) of the phenolic extracts from the trunk, fruits and leaves against multidrug-resistant Gram-positive and Gram-negative bacteria.

| Bacterial Strain       | Trunk (MIC mg/mL) | Fruit (Inhibition Zones (mm)) | Leaf (Inhibition Zones (mm)) |
|------------------------|-------------------|-------------------------------|-----------------------------|
| **Gram-positive**      |                   |                               |                             |
| *L. monocytogenes*     | 100 (10)          | 10 (9)                        | 10 (8)                      |
| *B. cereus*            | -                 | 25 (11)                       | -                           |
| *S. aureus*            | -                 | 25 (8)                        | 50 (9)                      |
| *S. epidermidis*       | -                 | 25 (10)                       | 25 (13)                     |
| *E. faecalis*          | -                 | -                             | -                           |
| *E. faecium*           | -                 | -                             | -                           |
| **Gram-negative**      |                   |                               |                             |
| *P. aeruginosa*        | -                 | 25 (12)                       | 75 (9)                      |
| *K. pneumoniae*        | -                 | 10 (12)                       | 10 (10)                     |
| *E. coli*              | -                 | 10 (9)                        | 25 (10)                     |
| *S. enteritidis*       | -                 | -                             | -                           |

The higher susceptibility zone (19mm), which was caused by the fruit extracts, was observed against *E. coli*. Overall, fruit extracts had the best antimicrobial efficacy, since they had effect against eight of the ten bacteria tested and showed lower minimum inhibitory concentration (MIC) values. In a study conducted by Chatzigeorgiou et al. (2017), the chemical composition of *Platanus orientalis* includes fatty acids, coumarins, terpenoids and mainly flavonoids and flavonoid glycosides. It was also reported that natural products isolated from the fruits of *P. orientalis* have antioxidant properties, active proteostatic mechanisms and can delay human cells senescence which could explain the high efficiency of the fruit extracts against the bacteria used in our study. Contrarily, the trunk extracts only had effect against *L. monocytogenes* with MIC of 100 mg/mL. There are several mechanisms trough which polyphenols can affect bacteria: by suppressing their virulence factors (e.g., inhibiting the biofilm production, decreasing the host ligand adhesion and neutralizing the microbial toxins), by inhibiting the synthesis of nucleic acids and the cell wall and by reducing the fluidity of the membrane. Furthermore, a synergistic effect may result from the interaction of different polyphenols. The antioxidant activity of phenols is due to the existence of hydroxyl groups and, therefore, modifications in the position of these groups could lead to changes in the antimicrobial properties [16]. The leaf extracts showed antimicrobial efficacy against six bacteria. Several studies showed that *Platanus* species leaves include flavonoids, tannins, pentacyclic triterpenoids and caffeic acid which have cytotoxic, cytostatic, antimicrobial and antiseptic properties [14].

Overall, the polyphenols extracted from *P. hybrida* demonstrated a notable antimicrobial activity against many antibiotic resistant bacteria, exhibiting better results when compared, with those reported for other *Platanus* species trees, such as *P. orientalis* [15].
4. Conclusions

The sensitivity of multidrug-resistant bacteria to natural phenolic compounds depends on bacterial species, the purity, and the polyphenol structure of the phenolics as well as the methods used for the experiments. We are aware that more studies, including in vivo experiments, should be undertaken to better clarify the molecular mechanisms underlying the protection of plane tree extracts against pathogenic bacteria. Nevertheless, the obtained results add evidence that these extracts can be an interesting source of phenolic compounds with antimicrobial activities that may provide assistance to antibiotics. Furthermore, the utilization of phenolic compounds extracted from the plane tree has significant importance within the circular economy principles of production and utilization of natural resources. However, in order to widely apply phenolic compounds as coadjuvants of antimicrobials, their safety and toxicity must be further investigated.

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