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

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Abstract: Bacterial resistance is a significant threat to public health and new classes of antibiotics and approaches to treatment are needed. It has been shown that natural plant-derived compounds can constitute a new alternative to fight microbial resistance but only a small number of studies were performed with antibiotic resistant bacterial strains. Therefore, in our work, we extracted phenolic compounds from the leaves, fruits and tree trunk of *Platanus hybrida* and evaluated their antibacterial activity against ten different multidrug-resistant bacteria. Two grams of each powder sample were weighed, and the extraction of the phenolic compounds was carried out with 100 mL of an ethanol and water (80:20) mixture by stirring for 2h. The extracts were redissolved in dimethyl sulfoxide (DMSO) and 100 mg/mL was the final concentration obtained. To test the antimicrobial activity of the phenolic compounds we used the Kirby-Bauer disk diffusion method. This method was performed against *Listeria monocytes*, *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 10 mg/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. hybrida*'s phenolic compounds may be an interesting therapeutic alternative. However, given that they haven't shown results against all bacteria, they only represent a possible start of the development of new classes of antibiotics.

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

1. Introduction

One of the most important scientific advances of the 20th century was the discovery of antibiotics. Antibiotics are substances with the capacity to selectively inhibit or kill microorganisms and, nowadays, they are often used in both human medicine and in livestock production [1,2]. Misuse of antibiotics has led to the development 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 stay active even when exposed to antimicrobial substances [1,3]. Bacterial resistance has been a problem since antibiotics were developed and it is are a growing cause of infections in hospitals and in the community [4]. Initially, the focus was on Gram-positive resistant bacteria. However, recently, the concern has moved to Gram-negative bacteria [5]. *Staphylococcus aureus* is Gram-positive bacteria that stands out for its ability to cause infections ranging from simple skin diseases to serious conditions such as bacteremia and endocarditis. Amongst Gram-negative bacteria, *Escherichia coli* is resistant to multiple therapeutic agents, such as carbapenemases and cephalosporins, and *Pseudomonas aeruginosa* is a bacterium with high virulence capacity. These opportunistic microorganisms are only specific examples of bacteria that can cause severe infections or even sepsis [6]. About 700 thousand death per year are due to the bacteria's ability to resist to the available antibiotics and, according to the World Health Organization (WHO), it is estimated that this number will grow substantially, reaching 10 million deaths per year by 2050. In addition, studies show that there will also be economic consequences worldwide: a 2.0 to 3.5% reduction of global gross domestic product (GDP) will be caused by the loss of 60 to 100 trillion dollars in economic production [3]. Consequently, chemical synthesis and isolation from natural products have been the main methodologies aimed at identifying new antimicrobial agents [6].

The use of plants for therapeutic reasons is an primitive custom that has been passed by one generation to the next [6,7]. Currently, and according to WHO, most populations of some Asian and African countries treat diseases with medicinal plants. Also, Europe and the USA have been increasing their use which evidences their therapeutic outcome [7]. Secondary metabolites are responsible for the therapeutic properties of medicinal plants: besides being physiologically important for plants, they additionally meddle with the pharmacological targets of humans and several other species [4]. Phenolic compounds, vitamin C, tocopherols and carotenoids are some of the natural antioxidants present in a large number of plants. These antioxidants can decrease oxidative damage and protect humans against Alzheimer, heart-disease and cancer. In addition to the antioxidant activity, plant-derived phenolic compounds have also exhibited activity against microbial agents and capacity to induce apoptosis [7]. *Platanus hybrida* Brot. (syn. *Platanus x acerifolia* (Ait.) Willd, *Platanus x hispanica* Mill. Ex Münchh), also known as London Plane, is a hybrid between *Platanus orientalis* L. (Oriental origin) and *Platanus occidentalis* L. (American origin) [8,9]. This specie belongs to Platanaceae family, flowers between March and April and gives fruit in the end of summer and autumn [8, 10]. It was first reported in the 17th century in Europe and since then, *P. hybrida* has been mostly used as an ornamental tree that offers shade and as a source of wood [9,10]. It is a very popular street tree both in Europe and in numerous parts of the world and it is commonly used because it provides a large number of ecosystem services, grows fast, has the ability to tolerate the urban microclimate and it is reasonably resistant to air pollution and soil compaction. Two of the main reasons for this tree to tolerate pollution are the morphological properties of the London Plane tree leave's which allows them to capture Particle Matter (PM) and its capacity to accumulate pollutants in its cortex [9]. Considering the current need to explore extra sources of compounds with antimicrobial properties, in this work, we extracted the polyphenols from leaves, fruits and trunk of this plane tree and investigated their ability to kill or inhibit several multidrug-resistant bacterial strains.

2. Materials and Methods

2.1. Plant Componentsl and Extract Preparation

The trunk, fruits and leaves were the three plane tree components used. Samples were collected in July 2020 in Trás-os-Montes and Alto Douro (Portugal). All of the components were separated, lyophilized and manually mill-powdered. Two grams of each powdered sample were weighted. The phenolic compounds were extracted with 100 mL of an ethanol and water (80:20) mixture by stirring for 2 h. Subsequently, all of the samples were centrifuged at 10,000 RPM for 15 min. Each supernatant was collected, filtered, and the solvent evaporated on a rotary evaporator at 40 °C under reduced pressure. Finally, for the analysis of antimicrobial activity, the obtained dry residues were weighted and redissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL. The extracts were stored under −20 °C until further analysis.

2.2. Antibacterial Activity

2.2.1. Bacterial Strains

The antimicrobial susceptibility was tested with six Gram-positive bacteria (*Listeria monocytes*, *Bacillus cereus*, *Enterococcus faecalis, Enterococcus faecium*, *Staphylococcus epidermidis* and *Staphylococcus aureus)* and four Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella enteritidis* e *Escherichia coli*). All of the bacterial strains were originally provided by the University of Trás-os-Montes and Alto Douro and University of La Rioja collections and then subcultured in brain heart infusion (BHI) agar (Oxoid, Basingstoke, UK) for 24 h at 37 °C. For the antimicrobial susceptibility assay we used Muller–Hinton (MH) agar (Oxoid, Basingstoke, UK).

2.2.2. Antimicrobial Susceptibility Test

Kirby–Bauer disk diffusion method was the chosen method for the antimicrobial susceptibility test. All of the bacterial strains were seeded in BHI agar plates and incubated overnight at 37 °C. Before the inoculation on MH plates, each colony was suspended in physiological solution to a turbidity equivalent to 0.5 McFarland standard. Five different concentrations of the extract's solutions (100, 75, 50, 25, and 10 mg/mL) were tested against the ten multidrug-resistant bacteria. These solutions were obtained by diluting the initial solution of 100 mg/mL with DMSO. Sterile blank disks with 6 mm of diameter were loaded with 20 μL of each extract concentration and placed on the inoculated MH plates, which were incubated for 24 h at 37 °C. The antimicrobial activity was evaluated with the measurement of the inhibition zones.

3. Results and Discussion

The evaluation of the antimicrobial activity of *P. hydrida*'s phenolic compounds was accomplished by using the Kirby-Bauer disk diffusion method and all of the inhibition zones were clear-cut. It is important to mention that, in our work and in opposite to most studies, we used strains typically resistant to multiple therapeutic agents that include not only antibiotics but also natural compounds. Table 1 is the summary of the results obtained for the minimum inhibitory concentration (MIC). As expected, different extracts exhibit different antimicrobial effects.

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 [12]. 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 [14].

Table 1. Minimum inhibitory concentration (MIC, mg/mL) and inhibition zones (mm) of the phenolic compounds extracted from the Plane tree's trunk, leaves and fruits against multidrug-resistant bacteria.

	MIC (mg/mL) (Inhibition zones (mm))		
Bacterial Strain	**Trunk**	**Fruit**	**Leaf**
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 [15]. 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. hydrida* 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 specie of the bacteria, the structure of the phenolic compound as well as the approach used for the experiment are just some examples of many things that affect the multidrug-resistant bacteria's response to natural phenolic compounds. For a better understanding of the molecular mechanisms responsible for the protection of the plane tree extracts against pathogenic bacteria, more studies are needed, including *in vivo* experiments. Nevertheless, the obtained results add evidence that these extracts can be a curious source of polyphenols with antimicrobial activity which may provide assistance to antibiotics. Moreover, the use the plane tree's polyphenols are an important step for the circular economy because it supports the production and consumption of natural resources. Nevertheless, in order to introduce phenolic compounds as antimicrobials coadjutor, it is required to investigate its toxicity and ensure that it is safe.

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Conflicts of Interest: The authors declare no conflict of interest.

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