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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: Multidrug-resistant bacteria are a significant threat to public health and new classes of antibiotics and approaches to treatment are needed. Several studies showed 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 hybrida* 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. Antimicrobial susceptibility assay was performed using Kirby-Bauer disc diffusion method and was tested against ten different bacteria: *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 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. hybrida*'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 is generating a problem affecting 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 researches 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, 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. Besides this, antimicrobial resistance will have economic implications: studies show 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 from 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 is increasing 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 which plays a critical physiological role in these organisms but also interferes 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 helps the human body to reduce oxidative damage and provide protection against heart disease, cancer and Alzheimer. Furthermore, it was already demonstrated that several plant-derived polyphenolic compounds exhibited important antimicrobial, antioxidant, anticancer and apoptosis-inducing properties [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 occidentalis* L. (American origin) and *Platanus orientalis* L. (Oriental origin) [8,9]. This specie belongs to Platanaceae family and it was first reported in Europa 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. hybrida* 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 of 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 Particule Matter (PM) due to the morphological characteristics of its leaves [9]. This way, 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 2h. 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 monocytes*, *Bacillus cereus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis)* and four multidrug resistant Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enteritidis* e *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 were 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 exhibit different antimicrobial effects. Nevertheless, it is important to notice that, unlike most studies, we used antibiotic resistant bacteria which have several mechanisms that confers 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]. It has often been reported that polyphenolic extracts are more efficient against Gram-positive bacteria [12]. Cell walls of Gram-negative represent a major barrier for the entry of phenolic compounds into cell cytoplasm due to the repulsion between lipopolysaccharide found in the surfaces of Gram-negative bacteria and phenols [13]. However, in this study, phenolic extracts 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 line with the obtained in other studies made with polyphenols extracted from winery by-products [14]. The resistance of phenolic compounds action by multidrug-resistant bacteria is not completely 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 [15].

Tabel 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.

	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*. In general, fruit extracts had the better antimicrobial efficacy, since they had effect against eight of the ten bacteria tested and showed lower MIC values. In a study conducted by Chatzigeorgiou et al. (2017), the chemical composition of *Platanus orientalis* is characterized by the presence of fatty acids, terpenoids, coumarins and especially flavonoids and flavonoid glycosides. It was also reported that natural products isolated from the fruits of *P. orientalis* exert antioxidant effects, active proteostatic mechanisms and delay human cells senescence which could explain the high efficiency of the fruit extracts against the bacteria used in our study [16]. Contrarily, the trunk extracts only had effect against *L. monocytogenes* with MIC of 100 mg/mL. The phenolic compounds can express their microbicide effect through different mode of action. These molecules can suppress several microbial virulence factors (e.g., by inhibition of biofilm formation, reduction of host ligand adhesion and neutralization of bacterial toxins), reduce the fluidity of membrane, inhibit the synthesis of nucleic acids and the cell wall or energy metabolism. In addition, the different phenolic compounds may have synergetic effect between them. The presence and number of hydroxyl groups in phenolic compounds is responsible for their antioxidant properties. Changes in the position of this group could play an important role in the antimicrobial activity and the interactions with cell membrane structures [17]. The leaves extracts showed antimicrobial efficacy against six bacteria. Several studies revealed that *Platanus* species leaves contain flavonoids, pentacyclic triterpenoids, tannins and caffeic acid and they have many pharmacological activities such as cytotoxic, cytostatic, antimicrobial and antiseptic effects [15].

Overall, the polyphenols extracted from *P. hydrida* exhibited a good antibacterial activity against several pathogenic bacteria resistant to antibiotics, presenting better results when compared, with those reported for other *Platanus* species trees, such as *P. orientalis* [16].

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 which may provide assistance to antibiotics. Furthermore, the utilization of phenolic compounds extracted from 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 antimicrobials coadjutor, their safety and toxicity must be further investigated.

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