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Research Article

Chemical profiles and antimicrobial activity from *Peperomia pellucida* tissues

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Abstract

The *Peperomia pellucida* species has been used as a medicinal herb due to its broad range of pharmacological properties. Although there is a number of a study with *P. pellucida*, there are no reports of antimicrobial activity for the leaf seedlings, roots, fruit and stems of *P. pellucida*. This study revealed that leaves, roots, fruit and stems *P. pellucida* exhibited antimicrobial activity against eight pathogens of clinical importance. The stem extract showed better antimicrobial potential when compared to the leaf, root and fruit extracts. The stem extract exhibited strong activity against bacteria *Escherichia coli* with MIC of 39 µg/mL and the fungus *Epidermophyton floccosum* with a MIC of 156.2 µg/mL. The seedling extract at 3 months exhibited strong activity against bacteria *Escherichia coli* with a MIC of 19.5µg/mL. The chemical profiles of extracts from leaves, stems, roots and fruit obtained by HPLC and GC/MS analysis were qualitatively and quantitatively different. The compounds 2,4,5-trimethoxycinnamic acid, 2,4,5-trimethoxystyrene and dillapiole were identified all extracts, except the 2,4,5-trimethoxystyrene which was not found in the root extract. These results contribute to the chemical and biological knowledge of *P. pellucida*, which is widely used in folk medicine.

1. Introduction

The Piperaceae family, genus *Piper* and *Peperomia*, was first described by Linnaeus in 1753 [1]. There are about 3000 species distributed in eight genera around the world, with 5 genera distributed among 500 species in Brazil. *Piper* and *Peperomia* are among the most studied of this family [2]. *Peperomia pellucida* is the most studied among the *Peperomia* species due to its wide distribution, applications in folk medicine and adaptation to different environments [2]. It germinates and grows easily in humid areas that are abundant in organic matter and protected from sunlight [2, 3]. Popularly known as "jabuti herb", "little heart", "frog tongue" and "glass herb" [4]; *P. pellucida* is widely used in folk medicine in the treatment of cough or sore throat, cardiac arrhythmias, abdominal pain, fatigue, headache, to control cholesterol levels, vaginal infections, renal infections, and for snake, scorpion and insect bites [5-7].

Pharmacological studies have revealed that crude extracts of *P. pellucida* exhibit antimicrobial, cytotoxic, anti-inflammatory, antioxidant, immunostimulating, anticancer, and antidiabetic activities [8-12]. Phytochemical studies of *P. pellucida* leaves report the presence of phenylpropanoids, flavonoids, tetrahydrofuran lignans and chromenes [13]. The essential oil of leaves of *P. pellucida* has been well investigated and it has shown the presence of phenylpropanoids, dillapiole and apiol as the major constituents [14]. But these studies with *P. pellucida* extracts have been carried out with the aerial parts of the plant, without differentiation. As part of the systematic study of plant compounds as antimicrobial

agents and considering that phytochemical studies using seedling tissues are limited, the present study was directed towards determining the chemical profiles and antimicrobial potentials of leaves, stems, roots and fruit of *P. pellucida*, with specific attention to the antimicrobial activity of young leaves of *P. pellucida.*

2. Materials and methods

2.1 Plant material

The leaves, roots, stems and fruit of *P. pellucida* were collected in March 2017 in the Atlantic Forest located around the Federal Rural University of Pernambuco (UFRPE- Recife –PE, 8°00'53.3"S and 34°57'04.7"W). The plant was identified by Dr. Margareth F. de Sales of the Department of Biology of UFRPE and a voucher specimen (Pipe-001-17) was compared with the collection of *exsiccates* belonging to Vasconcelos Sobrinho Herbarium of UFRPE. The seeds of *P. pellucida* were collected from adult species and sown to germinate in a mixture of commercial fertilized earth and sand. The seeds were cultivated in black plastic vases with a height of 5 cm, a surface of 5.8 cm and a lower diameter of 4.2 cm and a volume of 90 mL. Then, the pots were sealed with a transparent plastic and maintained in an environment under controlled temperature conditions at 28 ± 3 ºC, air humidity 56 ± 5%, artificial light and irrigated every 3 days.

2.2 Obtaining the extracts of P. pellucida

The leaves, roots, stems and fruits of *P. pellucida* were dried in an oven at 50 ºC for 48h and later crushed with a mortar and pestle until a fine powder was obtained. The dried macerated plant material was subjected to the cold maceration method for 48h to exhaustive extraction with dichloromethane by three times. All plant material was concentrated in a rotary evaporator under reduced pressure (40 ºC, ± 120rpm) to obtain the crude dichloromethane extracts of each tissue separately.

2.3 HPLC and GC/MS analysis

The crude extracts of *P. pellucida* (1.0 mg/mL at methanol) were analyzed in a gas chromatograph, Clarus 580 PerkinElmer, coupled to the mass spectrometer with a Clarus SQ8S model, elite-5MS column with dimensions of 30m x 0.25mm x 0.25µm, with system of split flow injection. The injection temperature was 250 °C and the samples were eluted on a programmed ramp of 40 to 280 °C at a rate of 25 °C / min. Helium gas was used as carrier gas at a rate of 0.56mL/min in split mode (1:30). To obtain the chromatographic profiles of *P. pellucida* extracts in HPLC, 2 mg of each extract had been previously diluted in 1 mL of CH_3OH and treated in a C18 reversed-phase silica solid phase extraction (SPE) cartridge (Sep-Pak) as the pre-purification step. Then, the crude extracts were analyzed with a chromatograph system coupled to a detector in the UV-Vis region with a diode array (HPLC-DAD) (Shimadzu LC10), eluted with mixtures of MeOH:H_2O in a polarity gradient at a flow of 1 mL/min. Analyses were performed on a Phenomenex ® C18 reversed-phase column (Luna C18 250 x 4.6 mm, 5 µm). The gradient started with 30% CH_3OH for 5 min, increasing to 50% CH_3OH in 15 min, reaching 100% for another 15 min and remaining at 100% for 5 min, returning to 30% CH_3OH in 5 min.

2.4 Chemical compounds

Dillapiole: $C_{12}H_{14}O_4$, m/z 222 (100); 208 (15); 176 (35); 148 (12) and 91 (8).

2,4,5-trimethoxystyrene: $C_{11}H_{14}O_3$, m/z 194 (100); 178 (72); 150 (33); 84 (82) and 62 (12).

2,4,5-trimethoxycinnamic acid: $C_{12}H_{14}O_5$, m/z 238 (100); 207 (70); 191 (19); 179 (32) and 163 (56).

2.5 Antimicrobial activity

The antimicrobial potential of the extracts of *P. pellucida* was evaluated against gram-positive bacteria *Staphylococcus aureus* (UFPEDA 02), *Bacillus subtilis* (UFPEDA 86), *Enterococcus faecalis* (UFPEDA 138) and gram-negative bacteria *Escherichia coli* (UFPEDA 224), *Klebsiella pneumoniae* (UFPEDA 396), as well as for the filamentous fungus *Epidermophyton floccosum* (UFPEDA 2563) and *Malassezia furfur* (UFPEDA 1320) and *Candida albicans* (UFPEDA 1007) yeast. The bacteria and fungi were acquired from the Microorganism Collection of the Department of Antibiotics at the Federal University of Pernambuco. The suspension of the microorganisms was standardized in distilled water by turbidity equivalent to McFarland's 0.5 tube, corresponding to a concentration of approximately 10^8 CFU/mL for bacteria and 10^7 CFU/mL for fungi. In order to determine the Minimum Inhibitory Concentration - MIC, 96-well multiples were used (CLSI, 2008; CLSI, 2010). The culture media employed for the MIC were

Sabouraud Agar (for fungi) and Mueller-Hinton Agar (for bacteria). Metronidazole (2.5 µg/mL) and Ketoconazole (2.5 µg/mL) were used as positive control, with dimethylsulfoxide (DMSO) for negative control. The analyses were performed in triplicate and the microplates were cultured at 37 °C for 18-24 h for bacteria and 30 °C for 48-72 h for fungi. After the culture period, the microplates were developed with the addition of 10 µL of 0.01% resazurin solution and incubated for 3 h (CLSI, 2010). The MIC was defined as the lowest concentration of the sample that inhibited the growth of the microorganism, with a final concentration of 2500 µg/mL.

3. Results and discussion

3.1 Chemical profiles

The chemical profiles of dichloromethane extracts from leaves, roots, stems and fruit of *P. pellucida* obtained by HPLC showed significant qualitative and quantitative differences (Fig. 1). Dillapiole phenylpropanoid was identified as a major constituent of the stems and fruit, while it was identified as a minor constituent in extracts of leaves and roots. 2,4,5-trimethoxycinnamic acid was identified as the major compound of leaf extract. The 2,4,5-trimethoxystyrene was identified in leaf, stem and fruit extracts. The compounds 2,4,5-trimethoxycinnamic acid (**1**), 2,4,5-trimethoxystyrene (**2**) and dillapiole (**3**) were identified based on the interpretation of their UV and MS spectra by comparison with their authentic standards. Chemical profiles for the leaves of seedlings aged 3, 4 and 5 months were similar to adult plant leaves with the presence of 2,4,5-trimethoxycinnamic acid, 2,4,5-trimethoxystyrene and dillapiole (Fig. 2). To our knowledge, this is the first report of 2,4,5-trimethoxycinnamic acid in the roots and stems of *P. pellucida* and as a chemical marker of young and adult leaves. There are many chemical studies with the aerial parts of *P. pellucida* revealed the predominance of flavonoids, phenylpropanoids and derivatives such as lignans [15]. The dillapiole and 2,4,5-trimethoxystyrene have been previously isolated from the aerial parts of *P. pellucida* [16]. The 2,4,5-trimethoxycinnamic acid has previously been reported only in the leaves of adult *P. pellucida*, while 2,4,5-trimethoxystyrene has been found in all parts of the plant as a minor constituent, including young and adult leaves [17]. Dillapiole has also been reported as the major component of the leaf and root oils from *P. pellucida* [18].

3.2 Antimicrobial activity

Crude extracts from different tissues (stem, root and fruit) of *P. pellucida* were subjected to antimicrobial assays against fungi and bacteria by determining the Minimum Inhibitory Concentration (Table 1).

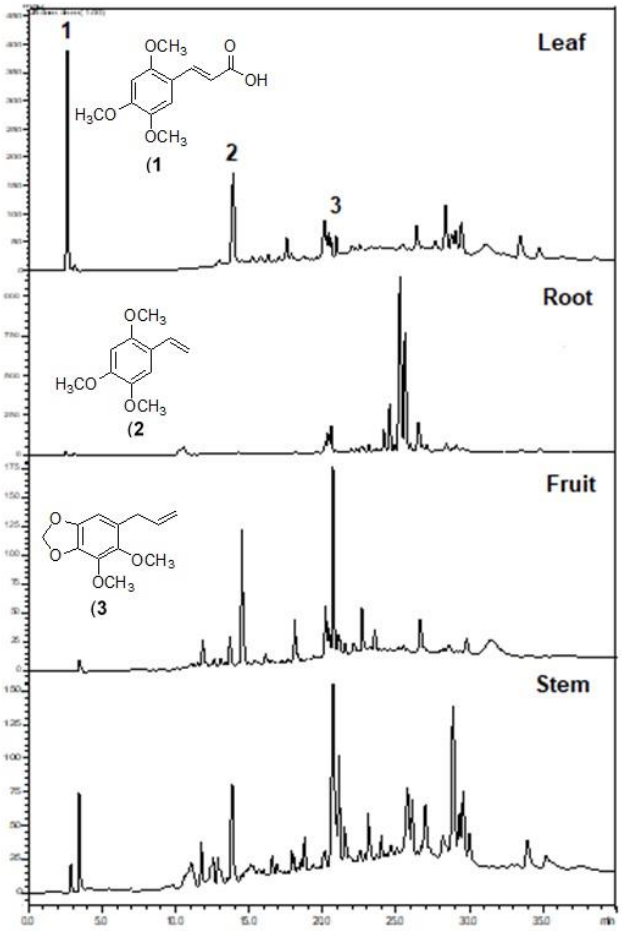

Figure 1. Chemical profiles obtained by HPLC of extracts from leaf, root, fruit and stem *P. pellucida*.

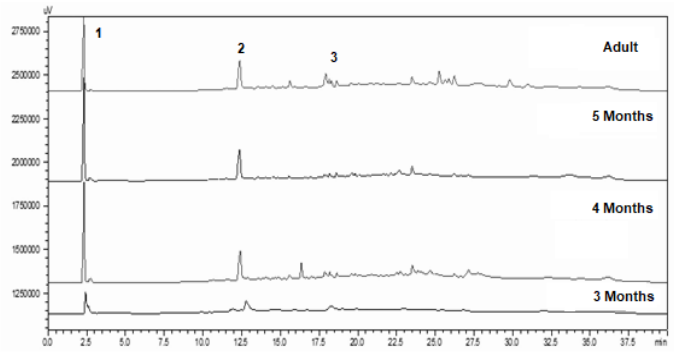

Figure 2. Chemical profiles obtained by HPLC of extracts from young and adult leaves of *P. pellucida*.

Table 1. MIC values at μg/mL of *P. pellucida*

Microorganisms	Stems	Roots	Fruit	Leaves			
				Adult	5 Months	4 Months	3 Months
S. aureus	312.5	2500	1250	2500	2500	2500	1250
B. subtilis	625	2500	1250	2500	2500	2500	2500
E. coli	39.0	2500	1250	2500	2500	2500	19.5
K. pneumoniae	625	1250	1250	2500	2500	2500	625
E. faecalis	312.5	1250	625	2500	2500	312.5	1250
C. albicans	625	625	2500	1250	1250	1250	1250
M. furfur	1250	1250	1250	2500	2500	2500	2500
E. floccosum	156.2	625	78.1	39.0	312.5	1250	1250

In general, the stem extract of *P. pellucida* had lower MIC values when compared to the values of extracts from the leaves, roots and fruit. The stem extract showed strong activity against *E. coli*, a gram-negative bacterium considered very resistant to antibiotics, with a MIC of 39 μg/mL, as well as for the fungus *E. floccosum* with a MIC of 156.2 μg/mL. This is a very significant since, although *P. pellucida* has been extensively studied, the result suggests that there should be more studies focused on evaluating the antimicrobial activity of differentiated extracts from fruit, roots and stems. There are previous reports of antimicrobial activity in studies with whole leaf and plant extracts or with essential oils, but there are no reports of extracts from separate tissues of *P. pellucida* [19]. In relation to the root of *P. pellucida*, there was better activity against the yeast *C. albicans* and the fungus *E. floccosum* with a MIC of 625 μg/mL. The fruit showed better activity against the fungus *E. floccosum* with an MIC of 78.1 μg/mL. The leaf extract exhibited strong activity against the fungus *E. floccosum* with a MIC of 39 μg/mL. In addition, MIC values for the leaves of seedlings aged 3, 4 and 5 months were determined, and all samples exhibited antimicrobial activity similar to adult plant leaves (Table 1). Phenylpropanoids with antimicrobial properties have been widely reported in plant essential oils, including the dillapiole and 2,4,5-trimethoxycinnami acid. Dillapiole was active against standard and multi-drug resistant strains *Staphylococcus spp.* with MIC of 1000 μg/mL [20]. Esters and amides derived 2,4,5-trimethoxycinnami acid showed antitumor, antiviral, antimicrobial and anti-inflammatory activities [21].

4. Conclusions

The extracts from the leaves, stems, roots and fruit of *P. pellucida* showed MIC values ranging from 19.5 to 2500 μg/mL for different types of gram-negative bacteria, gram-positive bacteria and fungi. The extract from the stems showed greater antimicrobial potential, with a broad spectrum for use in the treatment of infectious pathologies. The study also revealed that the leaves have antimicrobial activity regardless of the age of the plant. The results contribute to the chemical and biological knowledge of *P. pellucida*, a plant widely used in folk medicine, this being the first more complete study of the antimicrobial potential of *P. pellucida.*

This is the second biomonitored study during the development stages of a plant that revealed knowledge gaps in seedling chemistry. Chemical and biological studies with plants of different stages of development should receive more attention, as certain bioactive compounds are only found in young plants.

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Authors' contributions

Phytochemical study and biological activity: GBB; manuscript writing: CSR; Conceptualization, CSR.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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