Article

PHYTOCHEMICAL STUDY FROM THE AERIAL PARTS OF *Pavonia malacophylla* (MALVACEAE) AND EVALUATION OF ITS ANTIMICROBIAL POTENTIAL

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Pavonia malacophylla (Link & Otto) Garcke (Malvaceae *sensu lato*), commonly known as "malva-rosa" or "malva-veludo", is traditionally used to treat flu, cough, and heart pain. The crude ethanolic extract (CEE) of *P. malacophylla* was obtained through maceration with ethanol, concentrated using a rotary evaporator, and subjected to vacuum liquid chromatography with silica gel 60 as the stationary phase. Hexane, ethyl acetate, and methanol were used as mobile phases in increasing polarity gradients, yielding nine fractions. Chromatographic fractionation led to the isolation and identification of 18 compounds, including one alcohol, three steroids, three triterpenes, four pheophytins, six flavonoids and one phenolic acid. Among these, compounds **1** (decanol), **4a** (α-amyrin), **4b** (β-amyrin), **11** (5,8-dihydroxy-7,4'-dimethoxyflavone) and **16** (methyl-4-hydroxycinnamate), were identified for the first time in the genus *Pavonia*. Microbiological assays of the CEE, fractions, and isolated compounds were conducted against *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*, demonstrating antimicrobial activity at concentrations ranging from 250 to 400 μg mL^{-1}.

Keywords: *Pavonia malacophylla*; Malvaceae *sensu lato*; antimicrobial activity.

INTRODUCTION

The development and spread of microbial infections have generated worldwide concern.[1] The indiscriminate use and increase in consumption of antimicrobial agents, together with inadequate prescriptions, has contributed to the process of resistance to these drugs, whether through spontaneous mutations or the attribution of new genes that will be inherited by offspring.[2,3] Furthermore, the number of infections caused by multidrug-resistant microorganisms has amplified the spectrum of intractable diseases in the current context.[4]

From the moment penicillin was developed in the mid-1940s, the field of science turned to the search for antimicrobial derivatives from natural products.[5] Medicinal plants perform an essential role as the main therapeutic source used in folk medicine. Previous ethnic-medical-botanical studies form the basis for the discovery of new antimicrobials from these herbs.[6] Microbiological tests have demonstrated the high antimicrobial power of extracts, fractions, and isolated compounds, such as pheophytins, alkaloids, terpenoids and flavonoids, confirming the potential activity of these natural products.[7-11]

The Malvaceae *sensu lato* family comprises around 245 genera and 4465 species distributed throughout tropical and subtropical regions of the world.[12] Among the various genera belonging to this family, the genus *Pavonia* Cav. concentrates approximately 271 species distributed throughout the New and Old World, including Brazil.[13] Previous studies[11,14] report the prevalence of several classes of secondary metabolites in this genus, such as fatty acids, phenolics, steroids, terpenoids, flavonoids, pheophytins and hydrocarbons.

Pavonia malacophylla (Link & Otto) Garcke is popularly known as "malva-rosa" or "malva-veludo", distributed throughout Brazil. Its leaves and roots have been used to treat coughs and flu in the form of syrup, in addition to the use of its raw leaves to treat heart pain.[15,16] A preliminary phytochemical screening detected the presence of triterpenes, steroids, flavonoids, coumarins, tannins and alkaloids in the crude ethanolic extract from its aerial parts.[17]

Considering these aspects and the antimicrobial potential of species of the genus *Pavonia* linked to the shortage of phytochemical data on the species *P. malacophylla*, the present study aims to isolate and identify substances from their aerial parts and evaluate their antimicrobial activity.

EXPERIMENTAL

General and chemical procedures

Glass chromatographic columns were used for the isolation and purification of chemical constituents, packed with silica gel 60 (ASTM, 230-400 mesh, Macherey Nagel®), Sephadex LH-20 (Sigma-Aldrich®) or Amberlite XAD-2 (Sulpeco®).

Analytical thin layer chromatography (TLC) was carried out using plates impregnated with silica gel (Whatman®) to analyze the chromatographic profile of the compounds obtained in all processes and analyzed by ultraviolet radiation (UV) at wavelengths of 254 and 366 nm and revealed with diphenylboryloxyethylamine (NP) or

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p-anisaldehyde reagents, or by impregnating the plates in glass vats saturated with iodine vapors.

The identification or structural elucidation of the isolated compounds was carried out using spectroscopic methods (^{1}H and ^{13}C nuclear magnetic resonance (NMR)) and 2D techniques (HSQC (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond correlation)) using the BRUKER-ASCEND spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) and VARIAN operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) and 200 MHz (^{1}H) and 50 MHz (^{13}C), using deuterated solvents ($CDCl_3$, DMSO-d_6 and CD_3OD). Chemical shifts (δ) were expressed in parts *per* million (ppm) and coupling constants (*J*) in Hz.

Botanical material

The plant material (aerial parts) of *Pavonia malacophylla* were collected in the municipality of Santa Rita - Paraíba (7°7'42.473"S; 34°59'04.626"W) in June 2011. Botanical identification was carried out by Prof. Dr. Maria of Fátima Agra (CBioTec/UFPB) and an exsiccate was deposited in the Herbarium Prof. Lauro Pires Xavier (Agra 7038). This research was registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen - A568B8A).

Extraction and isolation of compounds

The aerial parts of *Pavonia malacophylla* were dehydrated in an oven with circulating air at an average temperature of 40 °C for 96 h. Then, the sample was crushed with the aid of a mechanical mill, providing 1050 g of powder. All material was macerated with 95% ethanol for 72 h. The extractive solution obtained was filtered and concentrated in a rotary evaporator under reduced pressure at 40 °C, providing 403.0 g of crude ethanolic extract (CEE).

A sample of CEE (200.0 g) was subjected to vacuum liquid chromatography (VLC) using a Buchner funnel coupled to a kitassato (1 L), and silica gel 60 as stationary phase and hexane (Hex), ethyl acetate (EtOAc) and methanol (MeOH) as mobile phases, alone or in binary mixtures in increasing order of polarity (Hex; Hex:EtOAc (9:1; 7:3; 1:1; 3:7); EtOAc; EtOAc:MeOH (9:1; 7:3; 1:1) obtaining nine fractions that were concentrated in a rotary evaporator and then analyzed by TLC. The Hex:EtOAc (9:1) fraction (6.0 g) was fractionated in column chromatography (CC) using silica gel 60 and Hex, EtOAc and MeOH following the previously described methodology, obtaining nine subfractions. The Hex:EtOAc (9:1) subfraction (930.0 mg) was chromatographed in CC following the same methodology, resulting in 44 subfractions that were analyzed by TLC. It was found that subfractions 14-20 and 26-31 were pure, being coded as compounds **1** (35.0 mg) and **2** (28.0 mg), respectively.

The Hex:EtOAc (7:3) fraction (3.5 g) was chromatographed in CC following the previous methodology, obtaining 107 fractions (10 mL each) that were analyzed by TLC. Fractions 47-53 and 64-69 were isolated, the compounds coded as **3** (13.0 mg) and **4** and (22.0 mg), respectively.

The Hex:EtOAc (3:7) fraction (3.0 g) was subjected to the previous methodology, obtaining 68 fractions that were analyzed by TLC. The fractions 11 and 13 were considered pure and were coded as compounds **5** (15.0 mg) and **6** (12.0 mg), respectively. The combination of fractions 14-24 (220.0 mg) submitted to a CC using Sephadex LH-20 as stationary phase and MeOH and MeOH:$CHCl_3$ (1:1) as mobile phases led to the isolation of compounds **7** (10.0 mg) and **8** (10.0 mg).

The 100% EtOAc fraction (5.0 g) was subjected to CC, using Sephadex LH-20 as the stationary phase and MeOH and MeOH:$CHCl_3$ (1:1) as mobile phases. From this process, 42 fractions were obtained, which were analyzed by TLC. Subfraction 26-31 was found to be pure and was coded as compound **9** (25.0 mg).

An aliquot of the EtOAc:MeOH (9:1) fraction (5.0 g) was chromatographed using the methodology described previously. From this procedure, 56 fractions were obtained and analyzed by TLC. The subfraction 44-51 (85.0 mg) was subjected to another chromatography, adopting the same methodology. From this, 20 fractions were obtained and analyzed by TLC. The subfractions 15-17 (11.0 mg) and 18-20 (5.0 mg) appeared as pure yellow powders, being then coded as compounds **10** (11.0 mg) and **11** (5.0 mg), respectively. The subfraction 19-43 (714.0 mg) was subjected to the same methodology as subfraction 44-51, resulting in 19 fractions. From this process, the subfraction 6-8 (33.0 mg) was obtained, which appeared pure when analyzed by TLC, in various solvent systems, being coded as compound **12** (33.0 mg).

The EtOAc:MeOH (1:1) fraction (7.0 g) was chromatographed in a CC on Amberlite XAD-2 using H_2O, MeOH, Hex, acetone and EtOAc as mobile phases. From this procedure, the 100% MeOH subfraction (2.8 g) was chromatographed on Sephadex LH-20 using MeOH and MeOH:$CHCl_3$ (1:1) as mobile phases. Successive chromatographic columns were performed using Sephadex LH-20 as the stationary phase and MeOH and MeOH:$CHCl_3$ (1:1) as mobile phases, resulting in fractions that were analyzed by TLC and then coded as compounds **13** (18.0 mg) and **14** (10.0 mg).

Another portion of the CEE (160.0 g) was dissolved in an ethanol:water solution (7:3) with a final volume of 1 L and homogenized with the aid of a mechanical stirrer for 2 h. Subsequently, the hydroalcoholic solution was subjected to liquid-liquid chromatography, using Hex, $CHCl_3$ and EtOAc (4 × 500 mL, each solvent), in increasing order of polarity, accompanied by TLC analysis, provided 34.0 g of hexane phase (HP), 37.0 g of $CHCl_3$ phase (CP), 13.0 g of EtOAc phase (AEP), in addition to the hydroalcoholic phase (HAP) (72.0 g).

An aliquot of CP (20.0 g) was subjected to CC using silica gel 60 as the stationary phase and Hex, EtOAc, MeOH and H_2O as mobile phases alone or in binary mixtures in an increasing degree of polarity, obtaining 67 fractions, which were concentrated in a rotary evaporator and combined through analysis in TLC. Fractions 15-16 and 29-30 were pure and were coded as compounds **15** (10.0 mg) and **16** (12.0 mg).

Antimicrobial activity assay

Test microorganisms

To evaluate antimicrobial activity, five strains of microorganisms were selected (*Staphylococcus aureus* ATCC 15656, *Streptococcus mutans* ATCC 25175, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 1106), obtained from the microbiological collection of the Oral Biology Laboratory, from the Health Sciences Center of the Federal University of Paraíba.

Determination of minimum inhibitory concentration (MIC)

Antifungal and antibacterial activities were determined using dilution assays in 96-well microplates in triplicate.

For the cultivation of bacteria and fungi, sterilized test tubes were used, where 0.6 mL of the bacterial/fungal inoculum was added with 7 mL of brain heart infusion (BHI) broth (for bacteria) and 7 mL of Sabouraud broth (for fungi). Then, the tubes were shaken and homogenized in the vortex, then taken to the oven at 35 ± 2 °C for 24 h for *S. aureus*, *S. mutans*, *E. coli* and *P. aeruginosa*, and 48 h for *C. albicans*. The cultivation of *S. mutans* was carried out using microaerophilia.

Stock solutions of extracts, fractions and compounds were prepared separately. These were weighed and mixed with a solution of 10% DMSO-d_6 and Tween 80 (5%) in a 1:1 ratio. The solutions were homogenized in the vortex, sonicated, and taken to an oven at 35 ± 2 °C.[18]

Secondary solutions SM1, SM2 and SM3 of the samples were prepared separately for three blocks of dilution series. For SM1, 1.5 mL of BHI broth was added with 1.5 mL of SM extracts/fractions/substances, described previously, both in the same proportion (1:1) (v/v), obtaining a final volume of 3 mL and an extract at a concentration of 500 µg mL^{-1}. For SM2, 2.5 mL of BHI broth were added with 0.5 mL of SM extract/substance, in the proportion (1:5) (v/v), obtaining a final volume of 3 mL and an extract at the concentration of 167 µg mL^{-1}. For SM3, 4.5 mL of BHI broth were added with 0.5 mL of extract/substance SM, in the proportion (1:9) (v/v), obtaining a final volume of 5 mL and an extract at the concentration of 100 µg mL^{-1}.

The preparation of the bacterial/fungal inoculum remained in an oven for 24 h of cultivation at 37 °C and 3 mL of the inoculum was removed from each tube and placed in Falcon tubes. Then, the tubes were centrifuged for 10 to 15 min, removing the supernatant and adding 240 µL of the inoculum with 4.8 mL of saline solution to the tube. Once the mixture was homogenized, it was placed in the oven for 1 h, vortexing every 15 min. After this rest, in an oven, the tubes were centrifuged again for 10 to 15 min and the supernatant was discarded and 2.4 mL of saline solution was added, homogenizing in the vortex.

The reading was then carried out on the spectrophotometer (OPTIMA) with an absorbance of 0.135 at a wavelength of 640 nm, corresponding to 1.6 × 10^8 CFU (colony forming unit) mL^{-1}, equivalent to 0.5 on the McFarland scale. After the incubation period, 35 µL of resazurin (100 µg mL^{-1}), prepared in an aqueous solution (1 mg, bring to volume 10 mL) was added to each hole, mixing the contents in the wells of the plate. Chlorhexidine was the antimicrobial used as a positive control.

RESULTS AND DISCUSSION

Identification of isolated compounds

Using 1D and 2D NMR spectroscopic methods, it was possible to identify and structurally elucidate 18 compounds from the aerial parts of *P. malacophylla*, including one aliphatic alcohol (**1**), three steroids (**2**) and two in mixture (**3a** and **3b**), three triterpenes, being two in mixture (**4a** and **4b**) and the compound **5**, four chlorophyll derivatives (**6**-**9**), six flavonoids (**10**-**15**) and one phenolic acid (**16**) (Figure 1).

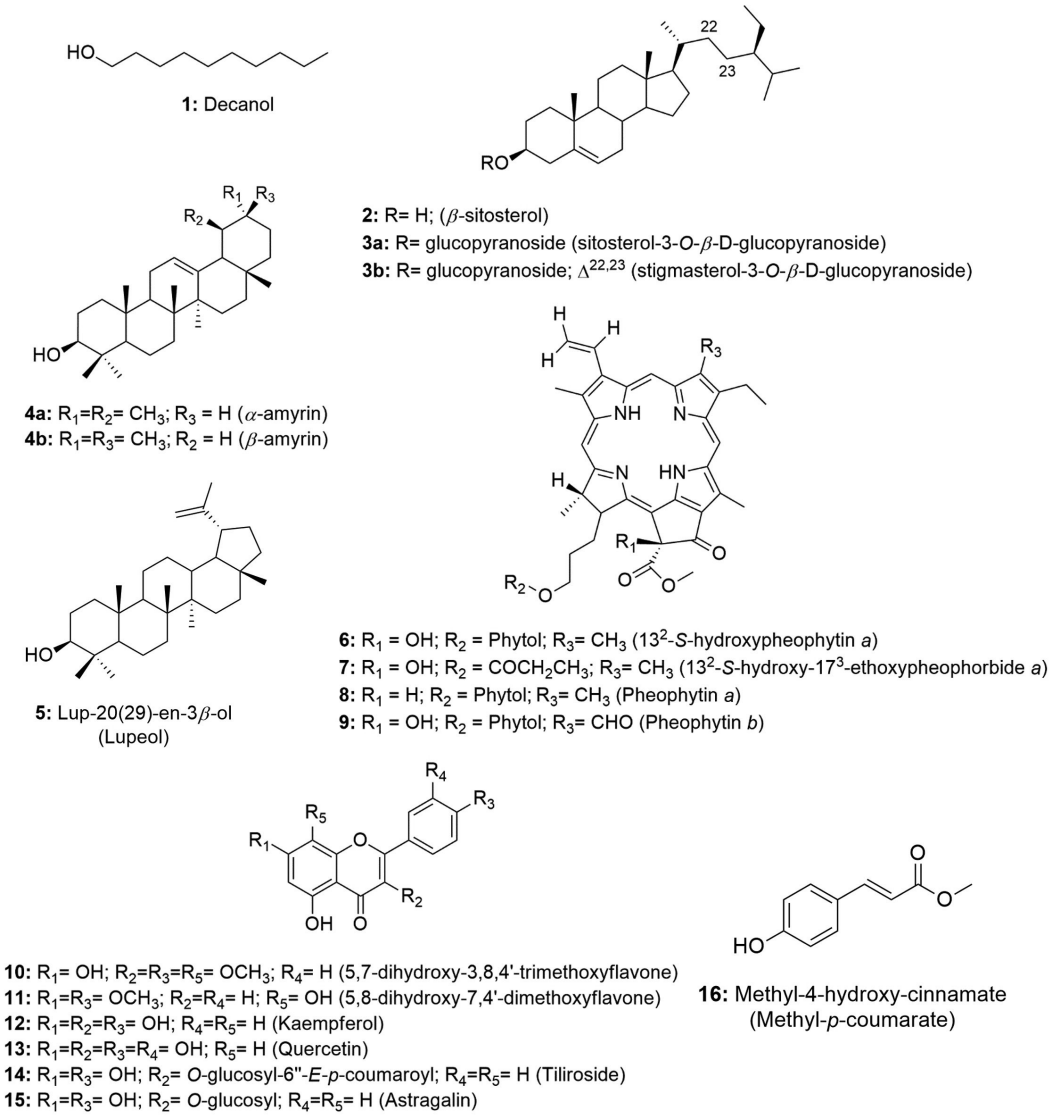

Figure 1. *Compounds isolated from the aerial parts of Pavonia malacophylla (Malvaceae sensu lato)*

*5,7-Dihydroxy-3,8,4'-trimethoxyflavone (**10**)*

Melting point of 174-175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.43 (s, 1H, 5-OH), 6.42 (s, 1H, s, H-6), 8.11 (d, 2H, *J* 10.0 Hz, H-2'/6'), 7.05 (d, 2H, *J* 9.0 Hz, H-3'/5'), 3.86 (s, 3H, 3-OCH_3), 3.99 (s, 3H, 8-OCH_3), 3.90 (s, 3H, 4'-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8 (C-2), 138.9 (C-3), 179.1 (C-4), 155.1 (C-5), 98.6 (C-6), 157.6 (C-7), 126.8 (C-8), 148.1 (C-9), 105.8 (C-10), 122.9 (C-1'), 130.2 (C-2'/6'), 114.4 (C-3'/5'), 161.9 (C-4'), 60.3 (3-OCH_3), 62.1 (8-OCH_3), 55.6 (4'-OCH_3). The ^{1}H and ^{13}C NMR data were in accordance with published data.[11]

*5,8-Dihydroxy-7,4'-dimethoxyflavone (**11**)*

Melting point of 269-270 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.43 (s, 1H, 5-OH), 6.86 (s, 1H, s, H-3), 6.55 (s, 1H, H-6), 8.11 (d, 2H, *J* 8.4 Hz, H-2'/6'), 7.12 (d, 2H, *J* 8.4 Hz, H-3'/5'), 3.90 (s, 3H, 7-OCH_3), 3.85 (s, 3H, 4'-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5 (C-2), 103.0 (C-3), 182.4 (C-4), 153.1 (C-5), 95.7 (C-6), 154.3 (C-7), 126.2 (C-8), 144.4 (C-9), 103.9 (C-10), 123.0 (C-1'), 128.5 (C-2'/6'), 114.5 (C-3'/5'), 162.4 (C-4'), 56.5 (7-OCH_3), 56.3 (4'-OCH_3), 55.6 (4'-OCH_3). ^{1}H and ^{13}C NMR data were in agreement with published data.[19]

*3,5,7,4'-Tetrahydroxyflavone (**12**)*

Melting point of 277-278 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.18 (d, *J* 2.0 Hz, 1H, H-6), 6.39 (d, *J* 2.0 Hz, 1H, H-8), 8.08 (d, 2H, *J* 8.9 Hz, H-2'/6'), 6.90 (d, 2H, *J* 8.9 Hz, H-3'/5'); ^{13}C NMR (125 MHz, CD_3OD) δ 148.0 (C-2), 137.1 (C-3), 177.3 (C-4), 162.5 (C-5), 99.3 (C-6), 165.6 (C-7), 94.4 (C-8), 158.2 (C-9), 103.7 (C-10), 123.7 (C-1'), 130.6 (C-2'/6'), 116.3 (C-3'/5'), 160.5 (C-4'). ^{1}H and ^{13}C NMR data were in agreement with published data.[20]

*3,5,7,3',4'-Pentahydroxyflavone (**13**)*

Melting point of 314-315 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.17 (d, *J* 2.0 Hz, 1H, H-6), 6.37 (d, *J* 2.0 Hz, 1H, H-8), 7.72 (d, 1H, *J* 2.0 Hz, H-2'), 6.87 (d, 1H, *J* 8.5 Hz, H-5'), 7.62 (dd, *J* 8.5 and 2.0 Hz, 1H, H-6'); ^{13}C NMR (125 MHz, CD_3OD) δ 148.0 (C-2), 137.2 (C-3), 177.3 (C-4), 162.5 (C-5), 99.2 (C-6), 165.6 (C-7), 94.4 (C-8), 158.2 (C-9), 104.5 (C-10), 124.1 (C-1'), 116.2 (C-2'), 146.2 (C-3'), 148.7 (C-4'), 116.0 (C-5'), 121.6 (C-6'). ^{1}H and ^{13}C NMR data were in agreement with published data.[8]

*Kaempferol 3-O-β-D-(6"-E-p-coumaroyl) glucoside (tiliroside) (**14**)*

Melting point of 254-255 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 6.11 (d, *J* 2.0 Hz, 1H, H-6), 6.27 (d, *J* 2.0 Hz, 1H, H-8), 7.96 (d, 1H, *J* 9.0 Hz, H-2'/6'), 6.79 (d, 1H, *J* 9.0 Hz, H-3'/5'), 5.23 (d, *J* 7.6 Hz, 1H, H-1"), 3.15-3.47 (m, H-2"/3"/4"/5"), 4.06 (dd, *J* 11.8 and 2.2 Hz, H-6^{a}"), 4.19 (dd, *J* 11.6 and 2.2 Hz, H-6^{b}"), 7.25 (d, *J* 8.6 Hz, H-2"'/6"'), 6.77 (d, *J* 8.6 Hz, H-3"'/5"'), 6.05 (d, *J* 16.0 Hz, 1H, H-α), 7.38 (d, *J* 16.0 Hz, 1H, H-β); ^{13}C NMR (50 MHz, CD_3OD) δ 159.2 (C-2), 135.1 (C-3), 179.3 (C-4), 162.8 (C-5), 99.9 (C-6), 165.8 (C-7), 94.8 (C-8), 158.2 (C-9), 105.5 (C-10), 122.6 (C-1'), 132.2 (C-2'/6'), 115.9 (C-3'/5'), 161.4 (C-4'), 104.0 (C-1"), 75.7 (C-2"), 77.9 (C-3"), 71.6 (C-4"), 75.7 (C-5"), 64.3 (C-6"), 127.0 (C-1"'), 131.1 (C-2"'/6"'), 116.7 (C-3"'/5"'), 161.1 (C-4"'), 114.6 (C-α), 146.5 (C-β), 168.8 (COO). ^{1}H and ^{13}C NMR data were in agreement with published data.[8]

*Kaempferol 3-O-β-D-glucopyranoside (astragalin) (**15**)*

Melting point of 162-163 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 6.12 (d, *J* 2.0 Hz, 1H, H-6), 6.29 (d, *J* 2.0 Hz, 1H, H-8), 8.03 (d, 2H, *J* 9.0 Hz, H-2'/6'), 6.87 (d, 2H, *J* 9.0 Hz, H-3'/5'), 5.14 (d, *J* 7.5 Hz, 1H, H-1"), 3.17-3.21 (m, H-2"), 3.41-3.44 (m, H-3"/4"/5"), 3.54 (dd, *J* 10.4 and 3.9 Hz, H-6^{a}"), 3.68 (dd, *J* 11.9 and 2.4 Hz, H-6^{b}"); ^{13}C NMR (125 MHz, CD_3OD) δ 158.5 (C-2), 135.3 (C-3), 178.9 (C-4), 162.7 (C-5), 101.3 (C-6), 170.3 (C-7), 95.8 (C-8), 158.8 (C-9), 104.4 (C-10), 122.8 (C-1'), 132.2 (C-2'/6'), 116.1 (C-3'/5'), 161.6 (C-4'), 104.6 (C-1"), 75.7 (C-2"), 78.0 (C-3"), 71.3 (C-4"), 78.3 (C-5"), 62.6 (C-6"). ^{1}H and ^{13}C NMR data were in agreement with published data.[21]

*Methyl-4-hydroxycinnamate (**16**)*

Melting point of 135-136 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* 8.7 Hz, 2H, H-2/6), 6.84 (d, *J* 8.7 Hz, 2H, H-3/5), 7.63 (d, *J* 16.0 Hz, 1H, H-7), 6.29 (d, *J* 16.0 Hz, 1H, H-8), 3.79 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 127.2 (C-1), 130.1 (C-2/6), 116.1 (C-3/5), 158.2 (C-4), 144.9 (C-7), 115.2 (C-8), 168.1 (C-9), 51.8 (OCH_3). ^{1}H and ^{13}C NMR data were in agreement with published data.[22]

Compound **1** was isolated as a viscous oil and identified as the aliphatic alcohol decanol, a compound produced by plants with ecological relevance that acts as a pesticide in the control of arthropod pests such as *Cimex lectularius* and as a plant growth regulator in agriculture.[23,24] Decanol showed good bactericidal activity due to its ability to damage the cell envelope of *Mycobacteria* species. Furthermore, the compound reduced biofilm formation by *M. smegmatis* at concentrations of 0.1 and 0.2 mM, lower than its MIC which was 0.4 mM.[25] This compound was previously reported in *Helicteres velutina* and *Helicteres eichleri*, species of Malvaceae *sensu lato*[26,27] and for the first time in the genus *Pavonia*.

Compound **2** showed melting point of 141-142 °C and was identified as β-sitosterol and compounds **3a** and **3b** were identified as a mixture of two glycosylated phytosteroids: sitosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranoside, respectively. These compounds are widely found as components of plant cell walls and membranes. Some activities are described in the literature[28-32] for the compounds, such as anti-inflammatory, analgesic, anthelmintic, antimutagenic, hypocholesterolemic, antidiabetic, antioxidant and antimicrobial. These compounds are widely found among species of the Malvaceae *sensu lato* family,[27,33] but isolated for the first time in the species *P. malacophylla*.

^{1}H NMR analysis of compounds **4a** and **4b** showed absorptions for two olefinic hydrogens at δ_H 5.16 (t, *J* 3.0 Hz) and δ_H 5.10 (t, *J* 4.0 Hz), indicating the presence of double bonds, suggesting that the compounds would be two triterpenes with unsaturation between C-12 and C-13. The hypothesis that it would be a mixture of triterpenes was corroborated by the characteristic signs of non-hydrogenated olefinic carbons at δ_C 145.1 (C-13) and monohydrogenated carbons at δ_C 121.6 (C-12) of triterpenes from the oleanane series, as well as the signs of non-hydrogenated olefinic carbons at δ_C 139.5 (C-13) and monohydrogenated at δ_C 124.3 (C-12) of ursane series triterpenes. Furthermore, it was shown that C-13 of the ursane series (δ_C 139.5) was more protected than C-13 of the oleanane series (δ_C 145.1), a fact due to the γ-gauche effect of methyl C-29 in the C-13 of the ursane series. Thus, compounds **4a** and **4b** were identified as a mixture of triterpenes from the ursane (α-amyrin) and oleanane (β-amyrin) series, respectively, isolated for the first time in the genus *Pavonia*.[34,35] A literature[36] reports for these triterpenes various activities such as anti-inflammatory, gastroprotective, anti-allergic, and antinociceptive.

The analysis of ^{1}H and ^{13}C NMR spectral data compared with literature data allowed us to verify that compound **5** would be lup-20(29)-en-3β-ol, known as lupeol,[37] which showed a melting point of 215-216 °C. This compound is widely distributed in the plant kingdom, including in species of Malvaceae *sensu lato*, such as *Herissantia tiubae*, *Helicteres eichleri*, *Waltheria viscosissima* and *Pavonia distinguenda*.[7,26,33,38] According to studies,[39-43] this

compound has great potential acting as an anti-inflammatory, anticancer, antidiabetic, cardioprotective, skin protective, antiprotozoal, antimicrobial, nephroprotective, antiangiogenic and hypocholesterolemic agent.

The ^{1}H and ^{13}C NMR spectral data of compounds **6**, **7**, **8** and **9** indicated that they are chlorophyll derivatives. Comparisons with literature data led to identifying the compounds as 13^2-*S*-hydroxypheophytin *a* (**6**),[44] 13^2-*S*-hydroxy-17^3-ethoxypheophorbide *a* (**7**),[45] pheophytin *a* (**8**),[10] and pheophytin *b* (**9**),[44] previously reported in species of Malvaceae *sensu lato*: *Wissadula periplocifolia*, *Sida rhombifolia*, *Sida galheirensis*, *Sidastrum micranthum*, *Helicteres velutina*, *Pavonia glazioviana*.[9,11,27,45,46]

The analysis of one and two-dimensional ^{1}H and ^{13}C NMR spectral data, together with comparisons with literature data,[8,11,19] allowed us to identify compound **10** as 5,7-dihydroxy-3,8,4'-trimethoxyflavone and compound **11** as being 5,8-dihydroxy-7,4'-dimethoxyflavone (7,4'-di-*O*-methylisoscutelarein), flavonoids previously isolated in species belonging to the Malvaceae *sensu lato* family.

The antimicrobial activity of flavonoids has been suggested according to their chemical structure, especially the number and positions of methoxyl and hydroxyl groups. The effect of *O*-methylation on flavonoids has been reported in the literature with reduced reactivity of hydroxyl groups and increased lipophilicity of these compounds, thus promoting an improvement in antimicrobial activity.[47,48]

Compounds **12**, **13**, **14** and **15** were isolated as yellowish powders. Their ^{1}H and ^{13}C NMR spectra showed signals in the region of aromatic hydrogens compatible with flavonoids, making it possible to identify them as 3,5,7,4'-tetrahydroxyflavone (kaempferol) (**12**), 3,5,7,3',4'-pentahydroxyflavone (quercetin) (**13**), kaempferol 3-*O*-β-D-(6"-*E*-*p*-coumaroyl) glycoside (tiliroside) (**14**) and kaempferol 3-*O*-β-D-glucopyranoside (astragalin) (**15**).

The astragalin was treated against fungal cells producing good antimicrobial and significant antibiofilm activity. Astragalin affected the integrity of the fungal cell membrane and had no cytotoxic effect on human gingival fibroblast cells. It also worked to reduce the expression of genes that encode efflux pumps (CDR1), and when combined with an antifungal drug, it increased its concentration inside cells, making it a therapeutic option for candidiasis.[49]

Compound **16** was isolated as a white powder. Its ^{1}H NMR spectrum exhibited signals in the region of aromatic hydrogens, with two doublets at δ_H 7.42 and δ_H 6.84 (*J* 8.7 Hz) with an integral for two hydrogens each and with an *ortho* coupling constant, suggesting the presence of an AA'BB' aromatic system.[50] Two other doublets were evidenced in δ_H 7.63 and δ_H 6.29 (*J* 16.0 Hz) with an integral for one hydrogen each, equivalent to the pair of hydrogens linked to the olefinic carbons and *trans*-type coupling constants, signals attributed to the hydrogens H-7 and H-8 of the caffeoyl unit, respectively. A signal consistent with methoxylic hydrogens in δ_H 3.79 with an integral for 3H suggested this group as a substituent in its structure.[22]

When evaluating the expansions of the ^{13}C NMR spectrum, signals were evidenced for 10 carbon atoms. The signal at δ_C 158.2 was attributed to C-4, due to its deprotection in relation to other aromatic carbons, due to the electronegative effect of the hydroxyl.[51] Absorptions at δ_C 127.2 and δ_C 168.1 were consistent with the quaternary carbons C-1 and C-9, while signals at δ_C 130.1 and δ_C 116.1 were attributed to the methine carbon sets of the AA'BB' (C-2/C-6 and C-3/C-5), respectively. The chemical shifts at δ_C 144.9 and δ_C 115.2 were compatible with those of the C-7 and C-8 *trans* coupling system. The only methyl carbon signal was evident at δ_C 51.8, thus confirming the proposal observed in the ^{1}H NMR spectrum. Two-dimensional HSQC and HMBC spectra of compound **16** confirmed the proposed molecule.[52]

The compilation of spectral data and comparison with literature data[10,52] allowed the identification of substance **16** as methyl-4-hydroxy-cinnamate (methyl-*p*-coumarate). This compound was previously isolated in the species *Hibiscus sabdariffa*[53] and *Abutilon indicum*,[54] but reported for the first time in the genus *Pavonia*. Few reports of activity with this phenolic acid show its potency against cells of the acute myeloid lineage in leukemias,[55] inhibition of melanoma formation[56] and antifungal activity against *Alternaria alternata*.[57]

Biological assay

The antimicrobial activities of all tested plant drugs are expressed in Table 1. For the *Escherichia coli* ATCC 25992 strain, the minimum inhibitory concentrations (MIC) of all fractions and extract were 400 μg mL^{-1} and for the isolated substances the MIC was 300 and 350 μg mL^{-1}. For *Staphylococcus aureus* ATCC 15656, only the EtOAc and 17^3-ethoxypheophorbide *a* fractions presented an MIC of 400 and 250 μg mL^{-1}, respectively. For *Candida albicans* ATCC 1106, only the EtOAc:MeOH (9:1) fraction showed an MIC of 400 μg mL^{-1}, when compared with the chlorhexidine standard.

The bioassays performed in this study were carried out using CEE, fractions and compounds isolated against bacterial strains and a yeast strain. The results obtained indicated that all tested samples had an inhibitory effect on the *Escherichia coli* strain (Table 1).

Table 1. Minimum inhibitory concentrations (MIC) of the extract, fractions and isolated substances of *Pavonia malacophylla*

Sample	Microorganism MIC / (μg mL^{-1})				
	Streptococcus mutans ATCC 25175	*Staphylococcus aureus* ATCC 15656	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 1106
CEE	–	–	–	400	–
Fr. Hex:EtOAc (9:1)	–	–	–	400	400
Fr. Hex:EtOAc (1:1)	–	–	–	400	–
Fr. EtOAc 100%	–	400	–	400	–
Fr. EtOAc:MeOH (9:1)	–	–	–	400	–
Fr. EtOAc:MeOH (1:1)	–	–	–	400	–
α and β-amyrin	–	–	–	300	–
17^3-ethoxypheophorbide *a*	–	250	–	350	–
Tiliroside	–	–	–	350	–
Chlorhexidine	25	15	8	70	64

CEE: crude ethanolic extract; Fr.: fraction; (–): growth of the microorganism.

The Hex:EtOAc fraction (9:1) promoted activity against the strain of *Candida albicans* and *Escherichia coli* with an MIC of 400 μg mL^{-1} for both microorganisms, and this action can be justified by the presence of other compounds in the sample, not having specificity between the bacterial and fungal strains tested.

The compounds α and β-amyrin (**4a/4b**), isolated from this fraction, were evaluated as a mixture of triterpenes enhancing antimicrobial activity against the *Escherichia coli* strain, with an MIC of 300 μg mL^{-1} when compared to its fraction from Hex:EtOAc (9:1) and its CEE, both with an MIC of 400 μg mL^{-1}. This result was also better when compared to the activity of just the triterpene β-amyrin against the same bacterial strains with an MIC of 10.0 mg mL^{-1}.[58]

Furthermore, in one study[59] the *in vitro* antibacterial activity against multi-resistant strains of *Staphylococcus aureus* of a mixture of α-amyrin and β-amyrin was demonstrated through synergistic action with antibiotics. In this same study with *in silico* tests, these compounds obtained responses due to the presence of the synergistic efflux mechanism, showing greater interaction with the MepA and NorA receptors, which are binding sites for conventional antibiotics, such as ciprofloxacin and norfloxacin, making this association a potential candidate for efflux pump inhibitor.

Many reports in the literature[60-63] still describe the antitumor, anti-inflammatory, antioxidant, hepatoprotective and antimicrobial potential of terpenoids. The good antimicrobial activity of pentacyclic triterpenes is linked to changes in the structure, morphology and functioning of bacterial cells, with difficulty in biofilm formation, adhesin production and gene expression.[64,65]

Gerola *et al.*[66] demonstrated the antimicrobial effect of chlorophyll-derived compounds, including pheophorbides. With diverse applicability, pheophytin *b* has shown potential for the production of biofilms, being a natural biological material used in applications such as electrochemical sensors.[67] Another study carried out by de Medeiros *et al.*[68] with pheophytin *b* demonstrated significant activity against strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at concentrations ranging from 25 to 50 μg mL^{-1}.

From this, it was decided to test the compound 17^3-ethoxy-pheophorbid *a* (**7**), since pheophytins have this antimicrobial power and share the same main skeleton, the porphyrin nucleus.

The EtOAc 100% fraction and the compound 17^3-ethoxy-pheophorbid *a* (**7**) tested showed activity of 400 and 250 μg mL^{-1}, respectively, against the *Staphylococcus aureus* strain, and both samples showed activity against *Escherichia coli* with MIC of 400 and 350 μg mL^{-1}, respectively[1]. A study carried out by Gomes *et al.*[46] further showed that pheophytin *a* also has a significant antimicrobial effect against the bacterial and fungal strains tested, where the minimum inhibitory concentrations ranged from 38 to 150 μg mL^{-1}.

The tested fraction EtOAc:MeOH (9:1) presented an MIC of 400 μg mL^{-1} against *Candida albicans*. From this fraction, a glycosylated flavone, tiliroside (**14**), was isolated, which showed good antimicrobial activity against a strain of *Escherichia coli*, with an MIC of 350 μg mL^{-1}.

Tiliroside has an amphipathic characteristic, due to the presence of a *p*-coumaroyl group in its chemical structure, ensuring greater lipophilicity.[69] This physicochemical characteristic determines its antimicrobial power, as it can bind to protein structures, such as efflux pumps produced by resistant bacteria, preventing other substances from diffusing to the outside of the microbial cell and not exerting their bactericidal/bacteriostatic activity.[70]

Hence to support this evidence, in our study *E. coli* was the only strain susceptible to all tested fractions and isolated substances < 400 μg mL^{-1} (Table 1). These results suggest that Gram-negative bacteria with the outer membrane containing lipopolysaccharide are potentially more susceptible than Gram-positive microorganisms.

Tiliroside isolated from the aerial parts of *Herissantia tiubae* demonstrated *in vitro* modulating activity of bacterial resistance against strains of *Staphylococcus aureus* (MIC = 256 μg mL^{-1}), modulating the activity of the tested antibiotics, which when administered concomitantly, reduced the MIC of norfloxacin and ciprofloxacin (16 times), lomefloxacin (four times) and ofloxacin (twice), and reduction to acriflavine (128 times), suggesting a possible inhibitor of the efflux pump in bacteria.[69]

Following recent classifications for antimicrobial activity, the extract, fractions and isolated compounds of *Pavonia malacophylla* reached levels of moderate activity (100-625 μg mL^{-1}) and with potentially useful activities.[71,72]

In this way, the antimicrobial activity presented by the extract, fractions and compounds isolated from *Pavonia malacophylla* can determine its main role in the environment, which is protection against pathogenic agents, thus reinforcing the need to intensify and deepen the mechanisms of action of these promising substances with antimicrobial potential.

CONCLUSIONS

The phytochemical studies with aerial parts of *Pavonia malacophylla* resulted in isolation and identification of 18 compounds, including one alcohol, three steroids, three triterpenes, four chlorophyll derivatives, six flavonoids and one phenolic acid.

The crude ethanolic extract, fractions, and four isolated compounds (α and β-amyrin, 17^3-ethoxypheophorbide *a* and tiliroside) were analyzed against the *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus* strains, showing activity in concentrations ranging from 250 to 400 μg mL^{-1}, thus providing an antimicrobial alternative in controlling infections. These results contributing to the chemotaxonomic and ethnopharmacological knowledge of the Malvaceae *sensu lato* family.

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AUTHOR CONTRIBUTIONS

JBLA (author), OCS, WAMQ, DAF, BFAR, MFA and MFVS carried out the phytochemical study and identification of the compounds. MFVS supervised the work. FCS performed the microbiological tests.

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