Phytochemical prospecting and biological activities of the floral extract from [*Impatiens walleriana* (Hook.)] (Balsaminaceae)

Wilker Urzeda Ferreira[1], Antonio Carlos Pereira de Menezes Filho[2], Porshia Sharma[3], Carlos Frederico de Souza Castro[2], Lenio Urzeda Ferreira[4] & Matheus Vinícius Abadia Ventura[1,2]

[1] UniBRAS University Center of Sudoeste Goiano, UniBRAS, Rio Verde, Goiás State, Brazil

[2] Goiano Federal Institute, IF Goiano, Rio Verde, Goiás State, Brazil

[3] Institute for Innovative Learning and Research (IILR) Academy, Indore, India

[4] Syngenta Seeds, Lucas do Rio Verde, Mato Grosso State, Brazil

Correspondence: Wilker Urzeda Ferreira, Centro Universitário do Sudoeste Goiano, UniBRAS, Rio Verde, Goiás State, Brazil. E-mail: wilkerurzeda@hotmail.com

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Abstract

Impatiens walleriana is a plant species that presents diversity and variety of colors among flowers. This plant species has wide distribution and is easily cultivable throughout the world including natural gardens, greenhouses and parks. This study aims at evaluating the floral extracts of *I. walleriana* in terms of their qualitative phytochemical constitution and antifungal activities on *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, as well as on the reduction of DPPH free radical and on the inhibition of acetylcholinesterase (AChE). Flowers were collected from two cultivars *Pink* and *White* of *I. walleriana* and the extract produced by maceration. The phytochemical qualitative assay was carried out using different reagents for determination by precipitation or colorimetric alteration. The antifungal test was carried out in *Petri* dishes with different concentrations of floral extract on *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum*. The DPPH reduction assay was performed by percentage and as standard antioxidant ascorbic acid. The acetylcholinesterase inhibition test was performed in percentage using *Electrophorus electricus*. Positive results were observed for phenolics, anthocyanins, organic acids, alkaloids, catechins, tannins, carboxylic acids, hemolytic saponins and sesquiterpene lactones. Fungal inhibition effect was better observed for *cv. Pink* with 10-26%, 6% and between 9-11% on *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum*, respectively. The DPPH reduction activity showed satisfactory results for *cv. Pink* 49% followed by *cv. White* 41%. And for inhibition of AChE *cv. Pink* with 30% and *cv. White* with 27%. The floral extracts of *Impatiens walleriana* show good results for the biological activities tested, especially for *cv. Pink*.

Keywords: *Impatiens* genus, alkaloids, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, AChE inhibition.

Prospecção fitoquímica e atividades biológicas do extrato floral de [*Impatiens walleriana* (Hook.)] (Balsaminaceae)

Resumo

Impatiens walleriana é uma espécie vegetal que apresenta diversidade na constituição de cores em suas flores. É uma espécie vegetal com ampla distribuição, sendo facilmente cultivada no mundo em jardins naturais, estufas e em parques. Este estudo teve por objetivo, avaliar os extratos florais de *I. walleriana* quanto a sua constituição fitoquímica qualitativa e atividades antifúngica sobre *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* e *Colletotrichum acutatum*, bem como, na redução do radical livre DPPH e na inibição da acetilcolinesterase (AChE). Flores foram coletadas de duas cultivares *Pink* e *White* de *I. walleriana* e o extrato produzido por maceração. O ensaio qualitativo fitoquímico foi realizado através de diferentes reagentes para determinação por precipitação ou alteração colorimétrica. O teste antifúngico foi realizado em placa de *Petri* com diferentes concentrações de extrato floral sobre *S. sclerotiorum*, *C. gloeosporioides* e *C. acutatum*. O ensaio de redução do

DPPH foi realizado por percentagem e como antioxidante padrão ácido ascórbico. O teste de inibição da acetilcolinesterase foi realizado em percentagem utilizando *Electrophorus electricus*. Foram observados resultados positivos para fenólicos, antocianinas, ácidos orgânicos, alcaloides, catequinas, taninos, ácidos carboxílicos, saponinas hemolíticas e sesquiterpenolactonas. Efeito de inibição fúngica foi mais bem observado para *cv. Pink* com 10-26%, 6% e entre 9-11% sobre *S. sclerotiorum*, *C. gloeosporioides* e *C. acutatum*, respectivamente. A atividade de redução do DPPH apresentou resultados satisfatórios para *cv. Pink* 49% seguido de *cv. White* 41%. E para inibição da AChE *cv. Pink* com 30% e *cv. White* com 27%. Os extratos florais de Impatiens walleriana demonstram bons resultados para as atividades biológicas testadas, em especial para *cv. Pink*.

Palavras-chave: gênero *Impatiens*, alcaloides, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, inibição da AChE.

1. Introduction

Several floristic plant species are being included in floriculture systems, ornamentation in parks and gardens. Within this broad floristic spectrum *Impatiens walleriana* Hook. f. popularly known as "*maria-sem-vergonha*, lollipop cherry red or write, and busy lizzie" is globally used due to its availability in varied color range. Although being native to tropical East Africa it is widespread from North to South of America, the Pacific Islands, China in particular (Taiwan), Australia and New Zealand (Mandle et al., 2010).

The genus *Impatiens* L., family Balsaminaceae A. Rich., is described in studies with important biological activities such as antioxidant, antimicrobial, anti-inflammatory and anti-proliferative. The species *Impatiens walleriana* is herbaceous and perennial having diverse flower morphology and colors ranging from pink to white, with early flowering, resistant to diseases and extreme sunlight (Anderson, 2006; Lai, 2015; Ghanbari et al., 2019).

Although being used for ornamentation purposes, the extracts of *I. walleriana* shows potential significance in biological activities such as antioxidant and antifungal, significant contents of phenolic compounds caffeic acid hexoside, *p*-coumaric acid hexoside, eryodictiol-*O*-hexoside, non-anthocyanin phenolic TPA, TFlav and TNAPC, anthocyanin phenolic pelargonidin-*O*-hexoside-*O*-deoxyhexoside-hexoside, Peonidin-*O*-hexoside-*O*-*p*-coumaroylhexoside, Malvidin-3-*O*-*p*-coumaroylhexoside-*O*-hexoside, Pelargonidin-*O*-*p*-coumaroyl-hexoside-*O*-acetyl-hexoside (Pires Jr. et al., 2021), and with potential for use in the food, pharmaceutical and cosmetic industries (Haider; Ullah, 2019).

In recent days, studies that characterize and relate the composition of edible flowers along different bioactive properties have been taken up. This study aimed to evaluate the floral extracts of *Impatiens walleriana var.* pink and white in biological activities on the inhibition of phytopathological fungi *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, antioxidant activity (DPPH) and on acetylcholinesterase inhibition (AChE).

2. Materials and Methods

2.1 Sample collection and preparation

Specimens of *I. walleriana* with pink and white flowers (Figure 1) were collected in May 2022 from a rural property in the municipality of Rio Verde, in the State of Goiás, Midwest Brazil. Vouchers were deposited in the Plant Systematic Laboratory and Herbarium at Goiano Federal Institute, Rio Verde, Goiás State, (HRV 1535 and 1536).

Immediately after harvesting, the flowers were washed thrice using water and dried with the help of absorbent paper. The petals were removed, stored, and separated according to their colour in plastic containers with 70% ethanolic solution. The extract was produced by maceration for 48 h. It was then filtered through qualitative filter paper and the filtrate was collected. The extract was reduced in a rotary evaporator and the samples prepared were frozen, freeze dried, and stored in airtight bottles protected from light in frozen at analysis.

Figure 1. *Impatiens walleriana cv. Pink* (**A**), and *cv. White* (**B**). Source: Authors, 2023.

2.2 Phytochemical prospection

Phytochemical analysis was performed for the main groups of special metabolites present; this was done using staining, and qualitative precipitation techniques. The floral extracts from *I. walleriana* were subjected to test for: alkaloids, anthocyanins, organic acids, reducing and non-reducing sugars, anthraquinones, catechins, depsides and depsidones, steroids, coumarins, tannins, phenols, flavonoids, polysaccharides, proteins, amino acids, purines, foaming and hemolytic saponins, organic acids, azulenes, and sesquiterpenolactones as proposed by (David et al., 2019).

2.3 Antifungal activity

The agar diffusion method was used to determine the antifungal activity on *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *C. acutatum* as described by Toigo et al. (2022) adapted. The strains used are: SS12-21, CG 16-21 and CA15-67 respectively, from the second author's mycological bank. The fungal strains were cultured at 20 °C for 10 days for *S. sclerotiorum* and 28 °C for 3-5 days for the other strains.

A mycelium disc with a diameter of 7 mm was transferred to the center of *Petri* dishes with a diameter of 10 cm containing sterile potato, dextrose and agar (PDA) medium. Different floral extracts concentrations were used, dissolved in 0.5% dimethylsulfoxide (DMSO) to render doses between 50-500 µL mL^{-1}, in each plate 500 µL of the concentration was pipetted. The plates were transferred to an incubator at 20 °C (10 days) and 28 °C (3-5 days), respectively. The diameter of the zone of inhibition was measured and recorded as an indicator of antifungal activity and expressed in percentage (%) using a digital caliper. The commercial reference fungicide Frowncide 500 SC was used as a positive control (dose of 10 µL mL^{-1}).

The DMSO emulsifier was also evaluated at the lowest dose under investigation (100 µL mL^{-1}). The agar diffusion assays applied against the three fungi were performed in quadruplicate. Mycelial growth was obtained daily until complete fungal growth separately on control plates. The experimental design was completely randomized.

2.3 Antioxidant activity

The antioxidant assay was performed using the methodology as described by David et al. (2019) adapted, in the reduction of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). DPPH stock methanolic solution, was prepared with a concentration of 40 µg mL-1 and kept refrigerated at -8 °C in a place free of light. The floral

extracts were diluted in methanol in a 1:5 ratio (*v/v*). Then, an aliquot of 0.8 mL of the solution was added to 0.2 mL of the DPPH stock solution. The mixture was then kept at rest protected from light for 30 min in a refrigerator at -4 °C. After this time, the sample was read in a UV-*Vis* spectrophotometer at a wavelength of 555 nm. Distilled water was used as a negative control, and a standard ascorbic acid solution was used as a positive control. The ability to reduce DPPH was expressed in percentage (%) of sequestration according to equation 1.

$$AA(\%) = 100 - \{[(Abs\ sample - Abs\ blank/Abs\ control)100]/Abs\ control\} \quad \text{Eq. 1}$$

Where: AA(%) = Abs absorbance of the sample; Abs absorbance of the blank, and Abs absorbance of the control.

2.4 Acetylcholinesterase inhibition

The colorimetric method for determining AChE inhibition followed as proposed by Miloševića et al. (2020). The enzyme conc. 0.09 U mL^{-1}, conc. 0.014 M and DTNB conc. 0.01 M were dissolved in conc. 0.1 M, pH = 8, and the diluted extracts conc. 1 mg mL^{-1} in phosphate buffer solution + 10% DMSO (*v/v*). The floral extract was serially diluted 40 µL prepared directly in a 96-well microplate, so that the concentration range in the final volume was between 0.4-400 µM.

The solutions were adjusted to 160 µL with phosphate buffer 0.1 M whit pH = 8, and the enzyme was added 20 µL. After 15 min of incubation in biochemical oxygen demand (B.O.D.) without photoperiod at 25 ºC, aliquots of DTNB 10 µL and AChE 10 µL were added to the wells. Then, the plates were homogenized, and the incubation continued for another 40 min. Absorbance was read at 405 nm, in a Elisa UV-*Vis* microplate reader. As a blank, phosphate buffer 180 µL and DTNB 10 µL, and AChE 10 µL solutions were used. Maximum enzymatic activity was obtained by replacing the latex sample with 10% DMSO phosphate buffer solution and the floral extracts absorbance by replacing the enzymatic solution with phosphate buffer. A solution of conc. 10 µM was used as a positive control (standard inhibitor). The percentage (%) of enzyme reaction inhibition was calculated according to equation 2.

$$AChE\ (\%) = [(A-B) - (C-D)]/(A-B)*100 \quad \text{Eq. 2}$$

Where: A, B, C and D are the absorbances of the maximum enzymatic activity, reaction blank, enzymatic activity in the presence of the floral extract sample and the color of the sample solutions, respectively. The AChE assay was performed in triplicate.

2.5 Statistical analysis

The results are presented as mean ± standard. Tukey's test was applied to assess significant differences between samples, throughout the work, the level of significance is 5%.

3. Results and Discussion

Floral extracts from *I. walleriana* have been observed to contain an interesting number of special metabolic groups with positive qualitative results for phenols, anthocyanins, organic acids, cathequins, tannins, carboxylic acids, hemolytic saponins and sesquiterpenolactones (Table 1).

Pandey & Tripathi (2014) and Shaikh & Patil (2020) consider that, after prior analysis of the qualitative composition of vegetables, it is necessary to quantify these groups of compounds by chromatography such as: gas chromatography, liquid chromatography, high-performance liquid chromatography, high-performance thin layer chromatography, etc.

For examples, flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Bhandary et al., 2020); saponins have hypotensive and cardiodepressant properties; glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Brian et al., 1985; Olaleye, 2007). Reducing and non-reducing sugars provide protection against solar irradiance; organic acids show bacterial and fungicidal activity; alkaloids activities, anticholinergic (Table 3), antihypertensive, antimalarial, antitumor, antitussive, antiviral, etc. (Duarte et al., 2014).

Table 1. Phytochemical prospection of the floral extracts *Impatiens walleriana cv. Pink* and *cv. White*.

Phytochemical class	Reaction	
	Pink	White
Phenols	+	+
Flavonoids	-	-
Anthocyanins	+	-
Organic acids	+	+
Azulenes	-	-
Alkaloids	+	+
Reducing sugars	-	-
No-reducing sugars	-	-
Anthraquinones	-	-
Cathequins	+	-
Coumarins	-	-
Depsides and depsidones	-	-
Steroids	-	-
Tannins	+	+
Polysaccharides	-	-
Proteins	-	-
Amino acids	-	-
Carboxylic acids	+	-
Purines	-	-
Foaming saponins	-	-
Hemolytic saponins	+	+
Sesquiterpenolactones	-	+

Note: (-) Absent. (+) Positive. Analysis performed on three replicates. Source: Authors, 2023.

The floral extract of *I. walleriana cv. Pink* showed a maximum inhibitory activity of 26% on *S. sclerotiorum* (Table 2) while *cv. White* with only 8%. *S. sclerotiorum* showed some sensitization activity, this is possibly due to the special metabolites compound groups described in (Table 1). For the anthracnose strains *C. gloeosporioides* and *C. acutatum* growth inhibition was only observed for *cv. Pink*. The commercial fungicide showed 100% inhibition of fungal growth evaluated at a concentration of 10 μL mL^{-1}.

Other works demonstrate aptitude on the use of floral extracts in the inhibition of *S. sclerotiorum* 20-66% and 8-91%%, *C. gloeosporioides* 9-56% and 81-100%, *C. acutatum* 38-77% and 4- 100%, and *R. stolonifer* 7-21% and 12-51% for *Tabebuia roseoalba* and *Jacaranda cuspidifolia*, respectively. Menezes Filho et al. (2021) verified discrete antifungal activity on *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum* evaluating the ethanolic floral extract of *Spathoglottis unguiculata* (Orchidaceae) 9-31%, 5-10% and 8-12% respectively.

It is suggested that the phytocompound groups of both floral extracts of *I. walleriana* must be separated by high performance liquid chromatography (HPLC) and tested separately in future studies inorder to verify which or which groups present antifungal activity on the tested phytopathogens.

Table 2. Antifungal activity of *cv. Pink* and *cv. White* floral extracts from *Impatiens walleriana* on agricultural fungal strains.

Strains	Pink extract – Concentrations in µL mL^{-1} (%)					
	50	100	200	300	400	500
S. sclerotiorum	0.00fA	10.26eA	16.84dA	22.79cA	24.59bA	26.59aA
C. gloeosporioides	0.00bA	0.00bB	0.00bB	0.00bB	0.00bC	6.18aC
C. acutatum	0.00cA	0.00cB	0.00cB	0.00cB	9.42bB	11.62aB
CV	7.83					
	White extract – Concentrations in µL mL^{-1} (%)					
S. sclerotiorum	0.00dA	0.00dA	0.00dA	4.67cA	7.31bA	8.70aA
C. gloeosporioides	0.00aA	0.00aA	0.00aA	0.00aB	0.00aB	0.00aB
C. acutatum	0.00aA	0.00aA	0.00aA	0.00aB	0.00aB	0.00aB
CV	34.56					

Note: Frowncide Fungicide 500 SC (100%) inhibition positive Control. Negative control DMSO (0%) inhibition. Source: Authors, 2023. Means followed by the same lowercase letters in the lines and uppercase in the row are statistically equal by Tukey's test at 5% significance. Source: Authors, 2023.

The floral extracts of *I. walleriana* demonstrate reducing activity on the free radical DPPH (Table 3) however, lower than the standard of ascorbic acid (vitamin C) with 96.48%[a]. Menezes Filho et al. (2021) describe for the floral extract of *Tabebuia impetiginosa* potential activity with an inhibition concentration (IC_{50}) = 21.18 µg mL^{-1}, which is a promising result, mainly in the production of cosmetic or food products with the ability to reduce free radicals. Kakimori et al. (2019) also obtained promising results from the flora extract of banana (Musa paradisiaca) with a 43.03% reduction equivalent to IC_{50} = 0.2765 mg mL^{-1}. In the AChE enzyme inhibition, both extracts showed promising results, especially for *cv. Pink* (Table 2). Important results were also described by Marques et al. (2013) evaluating the ethanolic extract of the flowers of *Bellis perennis* (Asteraceae f.) between 38.13 to 55.83%. Results greater than 30% are considered moderate and greater than 50% potent AChE inhibiting agents.

Table 3. Antioxidant activities in the reduction of DPPH and inhibition of acetylcholinesterase by *cv. Pink* and *White* floral extracts of *Impatiens walleriana*.

Extracts	DPPH (%)	AChE (%)
Pink	49.29b	30.92a
White	41.23c	27.06b
CV	2.05	2.00

Note: Means followed by the same lowercase letters in the columns in the are statistically equal by Tukey's test at 5% significance. Source: Authors, 2023.

4. Conclusions

The floral extracts of *Impatiens walleriana cv. Pink* and *White* have potentially able phytochemical groups having wide importance in agriculture, food, pharmacology and in bio-industrial use. Significant effects were observed mainly in the antifungal activities on the phytopathogens *Sclerotinia sclerotiorum* and *Colletotrichum acutatum*, as an antioxidant in the reduction of free radical DPPH and inhibition of acetylcholinesterase, especially for *cv. Pink*. However, future studies should be carried out by evaluating and elucidating by means of chromatography; the quantitative determination of each compound within the groups of phytocompounds observed and thus analyzing separately that which compounds are responsible for such activities.

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6. Auhors' Contributions

Wilker Urzeda Ferreira: study design, antifungal, antioxidant and acetylcholinesterase inhibition analysis, article writing, corrections and submission. *Antonio Carlos Pereira de Menezes Filho*: plant collection, specimen identification, production of floral extracts, antifungal analysis, free radical reduction activity and acetylcholine inhibition. *Porshia Sharma*: grammar correction of English and scientific literature. *Lenio Urzeda Ferreira*: grammar correction of English and scientific literature. *Matheus Vinícius Abadia Ventura*: supervisor, statistical analysis, writing and scientific corrections.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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