

## In Vitro Antioxidant and Antimicrobial Activities of Ethanolic Extracts from Whole Plants of Three *Impatiens* Species (Balsaminaceae)

### Abstract

**Context:** Plants of *Impatiens* genus are recognized source of extracts with antioxidant and antimicrobial activity, including *I. balsamina* (Sanskrit name: Tairini), a plant traditionally used in Asia to treat infections and inflammation. However, there is little information related to activities of whole plant extracts. **Aim:** To investigate the *in vitro* antioxidant and antimicrobial activities of the ethanolic extract from whole plant of *I. balsamina*, *I. hawkeri* and *I. walleriana*. **Methods:** Whole plant material of each species was macerated with ethanol (80% v/v). Phytochemical screening was applied for detection of different metabolites. Total phenolic content (TPC) and total flavonoids content (TFC) were determined using Folin-Ciocalteu and aluminum chloride methods respectively. *In vitro* antioxidant properties were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, potassium ferricyanide antioxidant reducing power (PFRAP) and oxygen radical absorbance capacity (ORAC) assays. *In vitro* antimicrobial activity of extracts was evaluated against *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC 19615), *Streptococcus pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10536), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) using microdilution assay for minimal inhibitory concentration (MIC) assessment. **Statistical Analysis Used:** Results were expressed as mean  $\pm$  SD. Data obtained were analyzed statistically using ANOVA (one-way) followed by Tukey's *post hoc* test. Correlation analyses were performed using Pearson correlation test. **Results:** *I. hawkeri* extract showed the highest phenolic content (TPC: 44.04 mg GAE/g; TFC: 55, 02 mg QE/g) and was the most antioxidant extract. *I. balsamina* extract was the most active against all Gram positive bacteria (MIC: 2.5-10 mg/ml) tested and over *C. albicans* (MIC: 10 mg/ml). This extract also showed the widest antimicrobial spectrum. **Conclusions:** The evaluated *Impatiens* whole plant extracts are also promising sources of antioxidant and antimicrobial agents.

**Keywords:** Antimicrobial, antioxidant, *Impatiens balsamina*, *Impatiens hawkeri*, *Impatiens walleriana*

### Introduction

Oxidative stress has been associated with a wide number of pathologies such as Alzheimer's disease, Parkinson's disease, atherosclerosis, myocardial infarction and cancer.<sup>[1,2]</sup> Therapy with exogenous antioxidants is considered a good option against such conditions.<sup>[2]</sup> Plant extracts can be used in such therapies and there is *in vivo* evidence that support its use to reduce oxidative stress marker levels.<sup>[3,4]</sup> The antioxidant activity of plant extracts has been widely related with the presence of phenolic compounds which can stabilize free radicals such as reactive oxygen species (ROS), which are responsible of oxidative damage at cellular level.<sup>[4,5]</sup>

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Meanwhile, bacterial and fungal infections are growing as public health problems at a global scale. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* are microorganisms for whom it is important to discover a new antibacterial agents.<sup>[6]</sup> *Staphylococcus epidermidis* has also gained attention due to the increase of cases of nosocomial infections related to this bacteria.<sup>[7]</sup> *Streptococcus pyogenes* is another pathogen which should be taken into account considering the high incidence of airway infections caused by this Gram-positive coccus.<sup>[8]</sup> Moreover, *Candida albicans* and *Aspergillus niger* are fungi for whom it is important to find new antifungal agents considering the development of resistance to current antifungal drugs.<sup>[9]</sup>

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Several plants of *Impatiens* genus have been identified as sources of extracts or metabolites with antioxidant or antimicrobial activities, especially against Gram positive bacteria.<sup>[10-12]</sup> For instance, *I. balsamina* is a medicinal plant used in Ayurveda which is a recognized source of quinones with antimicrobial activity, and it has been traditionally used in several countries of Asia, including China, India, Korea and Taiwan, for the treatment of infectious and inflammatory diseases.<sup>[11-15]</sup> However, few published studies consider the possibility of using the whole plant of *I. balsamina* and species of the same genus, such as *I. hawkeri* and *I. walleriana*, as sources of extracts with antioxidant and antimicrobial properties. Moreover, there is little by way of published literature related to *I. hawkeri* biological activities.

Furthermore, *I. balsamina*, *I. walleriana* and *I. hawkeri* are the most cultivated plants from *Impatiens* genus. These plants are widely exploited by horticulture industry around the world, since they are used as ornamental or decorative plants.<sup>[16]</sup> Considering the above, it is relevant to establish if the whole plant of these species can be used as a source of antioxidant and antimicrobial agents. Therefore, the *in vitro* antioxidant and antimicrobial activities of the ethanolic whole plant extracts of *I. balsamina*, *I. walleriana* and *I. hawkeri* were evaluated. In consideration of the limited information related with phytochemical constitution of these plants, especially of *I. walleriana* and *I. hawkeri*, phytochemical screening was also carried out.

## Methods

### Reagents

Gallic acid, quercetin, trolox, amphotericin B, RPMI-1640 media (Sigma-Aldrich), ceftriaxone disodium salt (USP), cation-adjusted Mueller-Hinton broth, potato dextrose agar, tryptic soy agar (BD Difco), sterile horse blood (courtesy of Clodomiro Picado Institute, University of Costa Rica, San José, Costa Rica). All other reagents and solvents were of analytical grade.

### Collection and authentication of plant material

Plants materials were collected in January 2017 in Grecia, Alajuela province, Costa Rica. Plants were identified and authenticated by Dr. Carlos Morales Sánchez and Dr. Luis A. Fournier Oraggi Herbarium, University of Costa Rica (USJ). Voucher specimens of each species were conserved under reference numbers USJ-110 988, USJ-110 989, USJ-111 059 for *I. balsamina*, *I. hawkeri* and *I. walleriana* respectively. Whole plant material (aerial parts and roots) of each species was dried at 50°C using an electric oven. Dried material was ground to get a maximum particle size of 2 mm. The milled material was stored in an appropriate container at room temperature until required for use.

### Extract preparation

The preparation of each extract was based on the procedure described by Kang *et al.*<sup>[12]</sup> with modifications. Dried milled material (75 g from each *Impatiens* species) was macerated with 750 ml of ethanol (80% v/v) under light protection at room temperature for two weeks. Then, the extract was collected, and the plant materials were macerated again with 1150 ml of the same solvent for three additional days, again under light protection at room temperature. Extracts from both maceration processes were combined and filtered through Whatman No. 4 filter paper. The combined extract was evaporated under vacuum at 40°C to remove the ethanol. Extracts were completely dried using a freeze-dryer. Freeze dried extracts were stored under light protection at -20°C until further analyses. Extracts were identified as IBE, IHE and IWE for *I. balsamina*, *I. hawkeri* and *I. walleriana*, respectively.

### Phytochemical screening

Extracts were tested for alkaloids, saponins, cardiac glycosides, terpenic compounds, phenols flavonoids, tannins, quinones, carbohydrates, reducing sugars, amino acids and soluble proteins according to Iqbal *et al.*<sup>[17]</sup> and Tiwari *et al.*<sup>[18]</sup>

### Determination of phenolic content

#### Total phenolic content

Total phenolic content (TPC) of each extract was determined using the Folin-Ciocalteu method adapted to 96-well microtiter plate according to the procedure described by Bobo-García *et al.*<sup>[19]</sup> The gallic acid calibration curve was constructed with a linearity range of 20-200 µg/ml. TPC values were expressed as milligrams of gallic acid equivalents (GAE) per gram of freeze dried extract (mg GAE/g). All determinations were carried out in triplicate.

#### Total flavonoid content

Total flavonoid content (TFC) of each extract was determined using the aluminium chloride method adapted to 96-well microtiter plate. An aliquot of 50 µl of methanolic quercetin standard solutions and test solutions of the extracts was added to separated wells of a 96-well microtiter plate (NUNC, Denmark). Then, 50 µl of sodium nitrite solution 6 g/l was added to each well. Five minutes later, 50 µl of aluminium chloride hexahydrate 22 g/l was added to each well. Later, the solution was left to stand for 6 minutes, after which 50 µl of sodium hydroxide 0.8 M was added to each well. The microtiter plate was stirred for 30 seconds at medium speed of the automatic microtiter plate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT). The absorbance was then measured at 510 nm. The calibration curve of quercetin was constructed with a linearity range of 20-60 µg/ml. TFC values were expressed as milligrams of quercetin equivalents (QE) per

gram of freeze dried extract (mg QE/g). All determinations were carried out in triplicate.

### Determination of *in vitro* antioxidant activity

#### 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay

DPPH free radical scavenging activity assay was carried out according to Kenny *et al.*<sup>[20]</sup> A dose-response curve for each freeze-dried extract and trolox (used as positive control) was constructed using triplicated determinations for each concentration level. The effective concentration of the extracts and trolox required to reduce the DPPH solution absorbance in 50% (EC<sub>50</sub>) was established by regression on the dose-response curve. EC<sub>50</sub> values were then reported in mg per ml.

#### Potassium ferricyanide antioxidant reducing power assay

Reducing power of the extracts was evaluated using the potassium ferricyanide antioxidant reducing power assay (PFRAP) adapting the method described by Berker *et al.*<sup>[21]</sup> to 96-well microtiter plate format. An aliquot of 20 µl from each standard solution of trolox and test solutions of the extracts, prepared using ethanol 96% v/v as solvent, was added to separated wells of a 96-well microtiter plate. Then, 130 µl of hydrochloric acid 30 mM, 10 µl ferric chloride hexahydrate 0.2% w/v, 30 µl of potassium ferricyanide 1% w/v and 10 µl of sodium dodecyl sulfate 1% w/v were added to each well in that order. The microtiter plate was shaken for 30 seconds at medium speed of the automatic microtiter plate reader. Later, the solution was allowed to stand for 40 minutes. The absorbance was then measured at 700 nm. The calibration curve of trolox was constructed with a linearity range of 20-100 µM. PFRAP values are reported as trolox equivalents (TE), in micromol, per gram of freeze dried extract (µmol TE/g). All determinations were carried out in triplicate.

#### Oxygen radical absorbance capacity assay

ORAC assay was carried out according to the procedure described by Kenny *et al.*<sup>[20]</sup> with minor modifications. Fluorescence decay from standard and sample solutions was recorded for 60 minutes. Trolox calibration curve was constructed with a linearity range of 20-60 µM. ORAC values were reported as micromol of trolox equivalents (TE) per gram of freeze dried extract (µmol TE/g). All determinations were carried out in triplicate.

#### Culturing of pathogenic microorganisms

The antibacterial activity of extracts was tested against *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC 19615), *Streptococcus pneumoniae* (clinical isolate, identified through VITEK 2 system, obtained from Medical Bacteriology Laboratory, College of Microbiology,

University of Costa Rica, San José, Costa Rica), *P. aeruginosa* (ATCC 9027) and *E. coli* (ATCC 10536). While antifungal activity was evaluated against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). Bacteria and fungi were cultured on tryptic soy agar plates (supplemented with horse blood at 5 % v/v for *S. pyogenes* and *S. pneumoniae*) and potato dextrose agar plates respectively. Fresh cultures of bacteria were incubated at 35 °C for 24 hours. *C. albicans* and *A. niger* were incubated at 25 °C for 48 and 72 hours respectively.

### Minimal inhibitory concentration (MIC) determination

#### Inoculum preparation

Colonies or spores were taken from fresh culture using a sterile inoculation loop and deposited into a flask containing 9 ml of sterile saline solution (0,9 % w/v NaCl). This solution was supplemented with Tween 20 at 5 % v/v for *A. niger* spores suspension. The transmittance of this initial inoculum was adjusted to 25 ± 1 % adding sterile saline solution. The initial inoculum from bacteria was adequately diluted with sterile cation-adjusted Mueller-Hinton broth (supplemented with lysed horse blood at 5 % v/v for *S. pyogenes* and *S. pneumoniae*) to get a final inoculum of 3x10<sup>5</sup>-7x10<sup>5</sup> colonies forming units per millilitre (CFU/ml).<sup>[22]</sup> While fungal inoculums were diluted to 5x10<sup>2</sup> – 2.5x10<sup>3</sup> CFU/ml, for *C. albicans*, and to 4x10<sup>3</sup> – 5x10<sup>4</sup> CFU/ml, for *A. niger*, using RPMI-1640 broth without sodium bicarbonate, supplemented with 3-morpholinopropane-1-sulfonic acid (MOPS) (0.165 M), L-glutamine and glucose at 2% w/v.<sup>[23]</sup> The adequacy of inoculum size from each microorganism was corroborated by plate count method.

#### Broth microdilution assay

Plates for MIC determination on each pathogenic microorganism were prepared according to the procedure described by Balouiri *et al.*<sup>[23]</sup> A stock solution of 200 mg/ml of each extract in dimethyl sulfoxide was prepared. This solution was serially diluted (twofold dilutions) on wells of microtiter plates using sterile broth to get a concentration range between 39 µg/ml and 20 mg/ml (each well containing 50 µl). Then, 50 µl of the final inoculum was added to get an extract concentration range between 19.5 µg/ml and 10 mg/ml. The final volume of each well was 100 µl. The plates were covered and incubated at 35°C for 20 and 40 hours for bacteria and fungi respectively. After incubation time, the MIC was determined turbidimetrically at 640 nm as the lowest concentration of extract that inhibited microorganism growth, i.e. the one that did not show turbidity. Cation-adjusted Mueller-Hinton broth was used for bacteria. This medium was supplemented with lysed horse blood (5%v/v) for *S. pyogenes* and *S. pneumoniae*. RPMI-1640 broth without sodium bicarbonate and supplemented with MOPS (0.165 M), L-glutamine and glucose (2% w/v) was used for fungal strains. Ceftriaxone



disodium salt and amphotericin B were used as controls. Each experiment was carried out in triplicate.

#### Minimal lethal concentration determination

MLC were determined by plate count method. A 50  $\mu$ l aliquot of wells with no growth in the MIC determination assay was subcultured. Tryptic soy and potato dextrose agar plates were used for bacteria and fungi respectively. The culture medium was supplemented with horse blood (5% v/v) for *S. pneumoniae* and *S. pyogenes*. Plates were incubated at 35°C for 24 hours for bacteria, while plates with fungus were incubated at 25°C for 48 hours. After incubation time, viable colonies were counted. MLC was defined as the minimal concentration of extract that kills 99.9% of tested microorganism.<sup>[23]</sup>

#### Statistical analysis

TPC, TFC, ORAC and PFRAP values are presented as mean  $\pm$  standard deviation (n = 3). Results obtained on each assay were compared using one-way ANOVA, followed by Tukey's *post-hoc* test. Differences at  $P < 0.05$  were considered statistically significant. EC<sub>50</sub> values were calculated by regression in the respective dose-response curve. Correlation analysis was performed using Pearson's correlation test. GraphPad Prism 5.0 (San Diego, California, USA) was used for data analysis.

### Results

#### Phytochemical screening

All extracts were positive for presence of phenols, tannins, flavonoids and terpenes, carbohydrates, reducing sugars, amino acids and soluble proteins. IBE and IHE were positive for quinones. Saponins were detectable only in IHE. All extracts were negative for alkaloids and cardiac glycosides [Table 1].

#### Phenolic content

IHE showed the highest TPC value base on the Folin-Ciocalteu assay (44.04  $\pm$  0.59 mg GAE/g). IWE gave the second higher TPC value (20.72  $\pm$  0.33 mg GAE/g), while IBE exhibited the lower value for this parameter (16.17  $\pm$  0.37 mg GAE/g). Based on aluminium chloride colorimetric assay, IHE showed the higher TFC value (55.02  $\pm$  1.10 mg QE/g), followed by IBE (4.98  $\pm$  0.27 mg QE/g) and IWE (3.97  $\pm$  0.20 mg QE/g). After ANOVA and Tukey's *post hoc* analyses, significant differences ( $P < 0.05$ ) on TPC and TFC values between extracts were found.

#### Determination of *in vitro* antioxidant activity

IHE showed the highest *in vitro* antioxidant activity according to the results of the three assays performed. It showed the lowest EC<sub>50</sub> value (0.10 mg/ml), the highest PFRAP value (98.49  $\pm$  3.03  $\mu$ mol TE/g) and the highest ORAC value (1528.13  $\pm$  67.95  $\mu$ mol TE/g) [Table 2].

Moreover, a significant correlation between TPC values and results obtained in antioxidant activity assays was found ( $r^2 > 0.956$ ;  $P < 0.05$ ). Similar results were obtained for the correlation between TFC values and results from antioxidant activity assays ( $r^2 > 0.950$ ;  $P < 0.05$ ).

#### Antimicrobial activity

Extracts showed MIC values between 2.5 mg/ml and 10 mg/ml against the susceptible microorganisms employed at the assays [Table 3]. IBE was the only extract to reach the MIC over *C. albicans*. IHE did not reach the MIC over *S. aureus* nor over *E. coli*; however, it was able to reduce their growth to the extent of 45% and 50% respectively at a concentration of 10 mg/ml. This was established by comparison of the turbidity obtained for growth control

**Table 1: Preliminary phytochemical screening of *Impatiens* whole plant extracts**

Constituent	Test	<i>Impatiens balsamina</i>	<i>Impatiens hawkeri</i>	<i>Impatiens walleriana</i>
Alkaloids	Dragendroff's	-	-	-
	Meyer's	-	-	-
	Wagner's	-	-	-
Phenols	Ferric chloride	+	+	+
	Lead acetate	+	+	+
Flavonoids	Shinoda	+	+	+
	NaOH	+	+	+
	Gelatin	+	+	+
	Salkowski's	+	+	+
Terpenes	Liebermann-Burchard	+	+	+
	Keller-Killiani	-	-	-
Cardiac glycosides	Foam test	-	+	-
Saponins	Bontrager's	+	+	-
Quinones	Molisch	+	+	+
Carbohydrates	Benedict	+	+	+
Reducing sugars	Ninhydrin	+	+	+
Amino acids	Xanthoproteic reaction	+	+	+

+: Present, -: Absent

**Table 2: Results from assays for *in vitro* antioxidant activity evaluation of *Impatiens* whole plant extracts**

Extract	EC <sub>50</sub> * (mg/ml)	PFRAP ( $\mu$ mol TE/g)	ORAC ( $\mu$ mol TE/g)
<i>Impatiens balsamina</i>	0.41 <sup>A</sup>	26.97 $\pm$ 1.24 <sup>A</sup>	784.35 $\pm$ 37.65 <sup>A</sup>
<i>Impatiens hawkeri</i>	0.10 <sup>B</sup>	98.49 $\pm$ 3.03 <sup>B</sup>	1528.13 $\pm$ 67.95 <sup>B</sup>
<i>Impatiens walleriana</i>	0.44 <sup>A</sup>	30.08 $\pm$ 0.57 <sup>C</sup>	902.58 $\pm$ 8.98 <sup>C</sup>

\*EC<sub>50</sub> for trolox was 2.73  $\mu$ g/ml. Data are shown as mean $\pm$ SD (n=3) except EC<sub>50</sub> values. EC50 was calculated by regression analysis on dose-response curves. Different letters in the same column indicate significant differences ( $P < 0.05$ ) between extracts. EC<sub>50</sub>: Effective concentration necessary to reduce the DPPH solution absorbance in 50%, PFRAP: Potassium ferricyanide antioxidant reducing power, ORAC: Oxygen radical absorbance capacity, TE: Trolox equivalents, SD: Standard deviation, DPPH: 2,2-diphenyl-1-picrylhydrazyl

**Table 3: Minimal inhibitory concentration and minimal lethal concentration of *Impatiens* whole plant extracts against pathogenic microorganisms**

Microorganism	<i>Impatiens balsamina</i>		<i>Impatiens hawkeri</i>		<i>Impatiens walleriana</i>		Control*
	MIC (mg/ml)	MLC (mg/ml)	MIC (mg/ml)	MLC (mg/ml)	MIC (mg/ml)	MLC (mg/ml)	
<i>Staphylococcus aureus</i>	2.5	10	>10	>10	5	>10	4
<i>Staphylococcus epidermidis</i>	10	>10	10	>10	10	>10	2
<i>Streptococcus pyogenes</i>	5	10	5	10	10	>10	0.03
<i>Streptococcus pneumoniae</i>	5	10	2.5	10	5	>10	0.06
<i>Escherichia coli</i>	>10	>10	>10	>10	>10	>10	0.06
<i>Pseudomonas aeruginosa</i>	>10	>10	>10	>10	>10	>10	16
<i>Candida albicans</i>	10	>10	>10	>10	>10	>10	0.5
<i>Aspergillus niger</i>	>10	>10	>10	>10	>10	>10	0.5

\*Ceftriaxone disodium salt and amphotericin B were used as control for bacterial and fungal strains respectively. MIC: Minimal inhibitory concentration, MLC: Minimal lethal concentration

wells (wells inoculated but not treated with extracts) and the inoculated wells treated with extracts. None of the extracts showed activity against *P. aeruginosa* nor against *A. niger* at the concentration range assayed. Only IBE and IHE showed bactericidal activity at the evaluated range of concentration over Gram positive bacteria. IBE reached the MLC at 10 mg/ml over *S. aureus*, *S. pyogenes* and *S. pneumoniae* while IHE came to a MLC of 10 mg/ml against *S. pyogenes* and *S. pneumoniae* [Table 3].

## Discussion

Different kinds of primary and secondary metabolites were detected in the evaluated extracts. A relevant result obtained from the phytochemical screening was the detection of quinones in IBE and IHE. The isolation of 2-hydroxy-1,4-naphthoquinone (lawsone), 2-methoxy-1,4-naphthoquinone and methylene-3,3'-bilawsone from *I. balsamina* has been reported and these compounds are recognized to be responsible for the antimicrobial activity exerted by extracts of this plant.<sup>[24]</sup> The presence of such kind of secondary metabolites has also been reported in other species from the genus *Impatiens* including *I. capensis*, *I. glandulifera* and *I. noli-tangere*.<sup>[25,26]</sup> Therefore, further investigation is necessary to determine the identity of quinonic compounds detected in *I. hawkeri*. Saponins were detectable in IHE. These types of secondary metabolites has been detected and isolated for other *Impatiens* plants including *I. capensis*, *I. siculifer* and *I. pritzellii* var *hupehensis*.<sup>[27-29]</sup>

Terpenes were detected in the three extracts. These compounds can contribute to antimicrobial effects of plant extracts. An example of this is spinasterol, this compound has been isolated from *I. balsamina* and it is responsible, together with 2-methoxy-1,4-naphthoquinone, for the antibacterial activity of this plant against *Helicobacter pylori*.<sup>[30]</sup> Phenols, tannins and flavonoids were also present in the three extracts investigated, these types of metabolites have gained attention due to their antioxidant and antimicrobial activities.<sup>[5,31]</sup>

Comparing the results obtained for determination of phenolic content of the extracts with published data,

we found that TPC values obtained from the evaluated extracts (TPC: 16.17-44.04 mg GAE/g) are higher than those reported by Su *et al.*<sup>[11]</sup> for *I. balsamina* stem extracts obtained with different solvents (TPC: 2.88-13.63 mg GAE/g). IBE and IWE showed TFC values in the range reported for extracts analysed by Su *et al.*<sup>[11]</sup> (TFC: 0.98-7.87 mg QE/g), while IHE showed a flavonoid content above that interval, i.e. 55.02 mg QE/g. Moreover, the TPC values of investigated extracts are in the range reported by Kang *et al.*<sup>[12]</sup> for freeze dried ethanolic extracts of leaves and stems from *I. balsamina* (TPC: 12.92-103.94 mg GAE/g). IBE and IHE showed TFC values in the interval of flavonoid content reported for those extracts (TFC: 4.28-104.28 mg QE/g).<sup>[12]</sup> IHE showed a higher TPC and TFC values than methanolic extract of whole plants from *I. textori* (TPC: 37.56 mg GAE/g; TFC: 19.84 mg QE/g) according to results reported by Yang *et al.*<sup>[32]</sup>

Phenolic compounds can stabilize free radicals, including ROS and reactive nitrogen species (RNS) implicated in oxidative stress conditions which can cause cellular damage and induction of inflammatory processes by different mechanisms including the activation of inflammasomes.<sup>[5,33]</sup> Phenols can stabilize free radicals by two main mechanisms, single electron transfer (SET) or by hydrogen atom transference (HAT).<sup>[5]</sup> These properties of phenolic compounds can explain the correlation observed between phenolic content (TPC and TFC) and results from antioxidant activity assays. These results are consistent with previous correlation reported between phenolic content and antioxidant activity of *Impatiens* plants extracts obtained from different anatomical parts.<sup>[10,12]</sup> This correlation is also reported for another kind of plant material extracts such as seaweeds and berries.<sup>[34,35]</sup> DPPH assay allows to evaluate the antioxidant activity of plant extracts by both SET and HAT mechanisms, PFRAP assay helps determine the reducing power of extracts through SET mechanism, while ORAC assay evaluates antioxidant activity in accordance with HAT mechanism.<sup>[36,37]</sup> Therefore, the assays performed allow to get information about antioxidant activity of the extract and to relate it to these principal mechanisms.

Considering this information and the experimental results, it is clear that IHE has the best *in vitro* antioxidant activity by both SET and HAT mechanisms [Table 2].

To the extent of our knowledge and according to the review of prior investigations, this paper is the first to report ORAC values for *Impatiens* species extracts. In addition, it is the first to evaluate the reducing power of extracts from plants of this genus in terms of Trolox equivalents through the PFRAP assay. Despite the differences in experimental conditions with previously published data, it is important to note that the analyzed extracts have shown ORAC values (784.35-1528.13  $\mu\text{mol TE/g}$ ) in the same concentration range of extracts with high antioxidant activity. The three extracts showed a higher ORAC value than acetic extract of strawberry (540  $\mu\text{mol TE/g}$ ) and ethanolic extract of blackberry (674.2  $\mu\text{mol TE/g}$ ).<sup>[38,39]</sup> IHE even showed a higher ORAC value than acetic extract of cinnamon (1256  $\mu\text{mol TE/g}$ ).<sup>[40]</sup> Therefore, this information supports the fact that *Impatiens* plants can be considered as a potential source of natural antioxidants. This is consistent with previous investigations in which the relevance of other plants of this genus as source of new antioxidants was demonstrated.<sup>[10-12]</sup>

Natural antioxidants can play an important role as anti-inflammatory agents in addition to their relevance for the prevention of different sicknesses linked with oxidative stress including cancer, cardiovascular illnesses and neurodegenerative diseases.<sup>[1,2,41]</sup> This can be explained by their free radical scavenging properties over ROS which in high levels can trigger oxidative stress and related inflammation.<sup>[33,41]</sup> Therefore, the relevant antioxidant activity exerted by EIB can be considered as a factor which supports the use of *I. balsamina* in traditional medicine of India, China, Korea and Taiwan to treat inflammatory illnesses such as rheumatism, nail inflammation and swelling.<sup>[12,13,15,42]</sup>

Moreover, the obtained results are consistent with prior published data which indicates that *Impatiens* species extracts are especially active against Gram positive bacteria.<sup>[12]</sup> Difficulties to find plant extracts with activity against Gram negative bacteria have previously been reported, and they are related with the presence of an outer membrane of lipopolysaccharides in Gram negative bacteria. This membrane represents an additionally permeability barrier for antimicrobial compounds from plant extracts, unlike Gram positive bacteria.<sup>[43]</sup> Antifungal activity of IBE also agreed with results of previous investigations which demonstrated that the extracts from *I. balsamina* were active against *C. albicans* and other pathogenic fungi. This fact is related to the presence of quinonic compounds such as 2-methoxy-1,4-naphthoquinone in this plant.<sup>[13,44]</sup>

*I. balsamina* is a medicinal plant used in several countries of Asia, including India, China and Korea as an antimicrobial

plant.<sup>[12,13,42,45]</sup> The traditional use of decoctions and infusions from leaves, stems, flowers and whole herb of *I. balsamina* has been reported for the treatment of local and systemic infections of bacterial and fungal origin.<sup>[12,13,46]</sup> Thus, the results of the present investigation support these traditional applications and demonstrate that *Impatiens* whole plant can be also used as a source of extracts with antimicrobial activity. Furthermore, the results suggest that the whole herb extracts from *I. balsamina* could be more active over Gram positive infections than those produced by Gram negative bacteria or fungi. This helps get information related to the spectrum of antimicrobial activity of *Impatiens balsamina* and inspires interest for the future *in vivo* evaluation of their effects in Gram positive bacteria infection models.

Evaluated extracts are positive for different groups of secondary metabolites which can contribute with exerted antimicrobial activity. Quinones can cause the inhibition of microorganism enzymes such as topoisomerases, which are essential to cellular survival and replication.<sup>[47,48]</sup> Polyphenols such as flavonoids can inhibit the microorganisms growth by aggregative effect on cells, this kind of compound is especially active against Gram positive bacteria. Tannins can inactivate bacterial membrane-bound proteins and have high affinity for iron which is essential cofactor in bacterial metabolism.<sup>[31]</sup> Terpenes can induce instability on bacterial membranes leading the leakage of intracellular content and it has been proposed that triterpenes induce inhibition of bacterial replication by blocking DNA synthesis.<sup>[49,50]</sup> Taking into account these facts, we suggest that the antimicrobial activity showed by the evaluated extracts is due by additive or synergic effects of different kinds of natural active constituents which can act by different mechanisms.

Bussmann *et al.*<sup>[51]</sup> consider that plant extracts have a strong antimicrobial activity when they show a MIC <5 mg/mL according to broth dilution assay. Accordingly, antimicrobial activity of the evaluated extracts range between strong and moderate against susceptible microorganisms. However, determined MIC are higher than values reported for other extracts of *Impatiens* plants. Szewczyk *et al.*<sup>[10]</sup> reported MIC values between 0.125 mg/ml and 1 mg/ml against the same bacteria and fungi species for extracts obtained from aerial parts of *I. balsamina*, *I. walleriana*, *I. balfourii*, *I. glandulifera* and *I. parviflora* using ultrasonication with methanol/acetone/water (3/1/1 v/v/v) as extraction method. Nevertheless, variances in ambient features between plant collection sites and between experimental conditions can contribute to differences obtained in comparison with published data.

Taking into account the growing published evidence which indicates the potential of *Impatiens* plants as a source of antimicrobial and antioxidant extracts, *I. hawkeri* and *I. walleriana* were also studied.<sup>[10-14,32]</sup> Thus, the present work also demonstrates the antimicrobial activity of



these other two species and reaffirms the importance of *Impatiens* as a genus for the screening of plants with the purpose of selecting extracts with significant antimicrobial and antioxidant activities. Additionally, the present work supports the future investigation of *I. balsamina*, *I. walleriana* and *I. hawkeri* for the development of herbal products with antimicrobial and antioxidant activity. Likewise, it gives an argument in favour to do further efforts for the isolation and the structural elucidation of their active components, especially those from *I. walleriana* and *I. hawkeri* for which there is little information.

## Conclusions

In summary, even if the investigated whole plants have shown a widely variety of secondary metabolites, further efforts must be done to define their identity. Particular attention should be paid to IHE and IWE due the limited information available about their active components which are responsible for the antimicrobial activity. The present work supports the view that whole plants from *Impatiens* genus are also potential source of extracts with important antioxidant and antimicrobial activities. *Impatiens hawkeri* is the most promising source of antioxidant extracts while *I. balsamina* has the best antimicrobial activity profile.

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## Conflicts of interest

There are no conflicts of interest.

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