

Antioxidant and Antimicrobial activity of *Impatiens walleriana* local to Malaysia

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Abstract

To study the antioxidant and antimicrobial activity of *Impatiens walleriana* local to Malaysia, against pathogenic bacterial strains. Total phenolic content, DPPH antioxidant, Disc Diffusion assay were performed to screen the samples of *Impatiens walleriana* for antioxidant and antibacterial activity against four Gram-negative and six Gram-positive bacterial strains. Zones of inhibition were measured and compared with 10 mg of penicillin as standard drug. Methanolic extract gives a higher amount of phenolic contents among all extracts 67.57 GAE g/kg. The DPPH radical scavenging activity of extracts ranged from 52-80% were found. Methanol extracts exhibit high scavenging activity among all extract were 80.4%. Methanol extract showcase high antibacterial activity against *E-coli* (16 mm) in disc diffusion assay. In conclusion present study demonstrate that *Impatiens walleriana* local to Malaysia have significant phytochemical and antioxidant activity.

Keywords: *Impatiens walleriana*, Antimicrobial, Antioxidant, DPPH

1. Introduction

Ornamental plants are the great repository of the powerful chemotherapeutics and can give a most significant wellspring of natural antimicrobials. Plants yield a wide assortment of secondary metabolites which have antifungal, anti-pest and antibacterial impacts and are of pharmaceutical importance. These antioxidants offered protective mechanisms against oxidant produced in living organisms such as reactive nitrogen species (RNS) and reactive oxygen species (ROS). During oxidation, reactive nitrogen species and reactive oxygen species are delivered as by product, which was thought to be the dynamic phenomenon to different types of life for their ordinary physiological purposes. However, most of the reactive nitrogen species and reactive oxygen species were produced as by product of cellular metabolism are viewed harmful to cell and tissue damage. The ROS, such as Nitric oxide, Superoxide anions and Hydroxyl radical inactivate enzymes by the degenerative process in the body. Inactivation of the enzyme leads to wreck the important cellular components and is assumed to be responsible for different types of diseases such as cancer, diabetic mellitus, AIDS, atherosclerosis, aging and hypertension. The family *Balsaminaceae* contains over 1200 species, dispersed throughout world tropic and subtropics regions [1]. *Impatiens walleriana* also called “*busy Lizzy*”, balsam or simply *Impatiens*, native to Malaysia, Indonesia, India, China and Eastern Africa. *Impatiens walleriana* often connected to moist and humid habitats, and hence can be found adjacent streams, shady forest and in gorges. Additionally, it can be found along roads, in secondary forest and other disseminated habitats [2]. *Impatiens walleriana* is the herbaceous perennial plant growing 15 to 60cm tall with broad lanceolate leaves 4 to 12 cm long, and 2 to 5cm broad. *Impatiens* as an eatable and patterned garden plant that has been utilized in traditional medicine as antimicrobials, [3] anti-anaphylactic [2], anti-inflammatory [4], anti-dermatitic [5], Antitumor [6,7] and anthelmintic [8]. Leaves and dry roots juice drank as abortifacient [9]. In the context of the discussion, a study was embraced first time to assess the antioxidant and antimicrobial activity of *Impatiens walleriana* against gram positive and gram negative bacterial strains in vitro.

2. Materials and Methods

2.1. Plant collection and identification

Plant materials were commercially purchased from botanical garden Cameroon Highland, Malaysia. Plant materials were identified by the taxonomist of University Malaysia Pahang, Malaysia. All fresh plant leaves (200g) were collected, rinsed with distilled water and kept under shade at room temperature for drying. Dry leaves were ground finely powered. Fifty grams of fine powder was soaked in 500 mL of Methanol, Chloroform. All solvent extract was blended for vigorous shaking and mixing, filtered by Whatman filter paper. After filtration, all solvent extract was completely evaporated by rotary evaporator; semi dry extracts were put in separate bottles and were stored at -20° C.

2.2. Bacterial strains

All strains were cultured on sterilized nutrient agar slants and sub-cultured on fresh agar media. All gram positive (*Enterococcus faecalis*, *Enterococcus*, *Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas*, *Klebsiella pneumoniae*) strains were procured from Faculty of Science and Industrial Technology, University Malaysia Pahang, Malaysia. All strains were preserved at 4° C.

2.3. Total phenolic content assay

The total phenolic content was measured according to previously described Folin-Ciocalteu method [10]. In detail, 0.1 mL of each sample dissolved in methanol was diluted with 4.6 mL glass-distilled water in a volumetric flask. Then, Folin-Ciocalteu reagent (0.1 mL) was added and 3 min later, 0.3 mL of sodium carbonate solution (0.25 mol/L)

was added. After 2 h, the absorbance was read at 765 nm using UV-Visible spectrophotometer (Shimadzu, Japan). The same assay was performed for Gallic acid (standard) solutions. The total phenolic content was expressed as Gallic acid equivalents (GAE g/kg dry weight extract). The experiment was conducted in triplicate.

2.4. Total antioxidant capacity (DPPH assay)

The DPPH radical scavenging assay was conducted with minor modifications, according to the previous study [11]. The extracts (0.5 mL) were added to 0.5 mL of a 0.004% (w/v) freshly made ethanol solution of DPPH. The mixture was mixed vigorously using a vortex and left to stand in ambient temperature in darkness for 20 min, then the absorbance was measured at 517 nm using a spectrophotometer. Inhibition of free radical DPPH in percent was calculated by the formula:

$$\text{Scavenging effect (\%)} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} is absorbance of control (containing all reagents, except the sample) and A_{sample} is the absorbance of each sample. All experiments were run in triplicate.

2.5. Antibacterial Activity by disc diffusion assay

The antibacterial activity was performed by disc diffusion method previously described by Bauer [12] bacterial cell suspension whose concentration was equilibrated to a 0.5 McFarland standard. A 100 μL aliquot of each bacterial suspension was spread on a Mueller Hinton agar plate. Sterile paper discs (6 mm diameter) were impregnated with 20 μL of each total extract and fractions dissolved in the solvent used for extraction at 125 mg/mL. The discs were allowed to dry and then placed on the inoculated agar. Discs with the solvent used for dissolution were used as negative control and 10 mg Penicillin discs were used as positive controls. The plates were incubated at 37°C for 24 hr. After the incubation time, the zone of inhibition was measured. The experiment was performed in triplicate.

2.6. Statistical analysis

All experiments were performed in triplicate, and statistical analyses were performed on Microsoft Excel 2010. All the data were given mean \pm standard deviation (SD). $P < 0.05$ was taken as significant with 95% confidence interval.

3. Results

3.1. Total phenolic contents

Total phenolic contents were found in a range of 64.89-67.57 GAE g/kg (Figure 1). The capability and correlation of the various solvent extract for total phenolic content, though, methanol extract gives a high amount of total phenolic content as compared to hexane and chloroform extract.

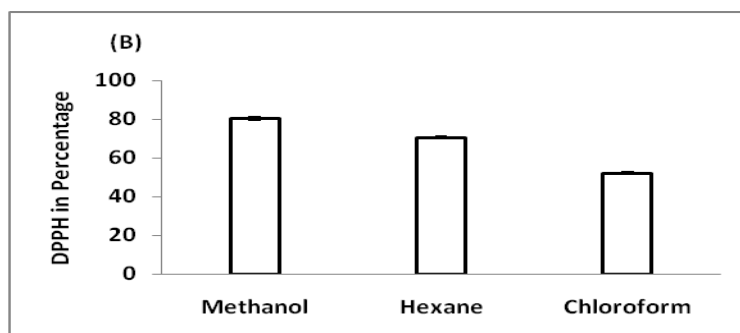


Figure 1: Total Phenolic Contents of *Impatiens walleriana* in different solvent Extract

3.2. The determinations of antioxidant activity

The determination of antioxidant activity of *Impatiens walleriana* methanol, hexane, and chloroform extract were utilized. A great number ornamental, aromatic, medicinal and other plants comprise chemicals compounds that show antioxidant properties. For antioxidant activity, different assays were used comprising scavenging activity of total phenolic content and DPPH assay was also measured (Figure 2). The DPPH radical is steady and free radical which is utilized effectively to evaluate free radical scavenging activity. Antioxidants neutralize DPPH by donating hydrogen or electron [13]. The DPPH radical's scavenging activity of various extracts of *Impatiens walleriana* plant extract shown in (Figure 2). The DPPH radical scavenging activity of extracts ranged from 52-80% were found. Methanol extracts exhibit high scavenging activity among all extracts were 80.4%, 70.6%, and 52.5% respectively as compared to control BHT.

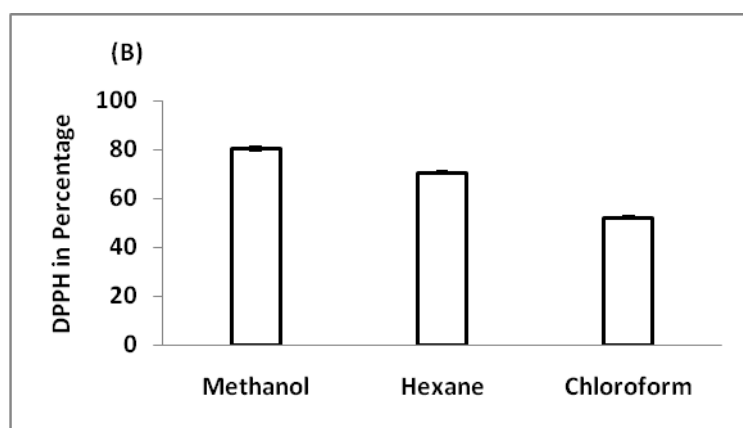


Fig 2: DPPH Assay for *Impatiens walleriana* in Methanol, Hexane and Chloroform Extracts.

3.3 Antimicrobial activity

Disc diffusion assays were performed to screen out the anti microbial activity of *Impatiens walleriana* extract against gram positive and gram negative bacterial strains. Methanol extract exhibited high antibacterial activity against *E. coli* (16 mm) followed by *Salmonella typhi*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (15 mm) (Figure 3). Additionally, chloroform extract was also found impervious to *Staphylococcus aureus* in comparison with methanol extract.

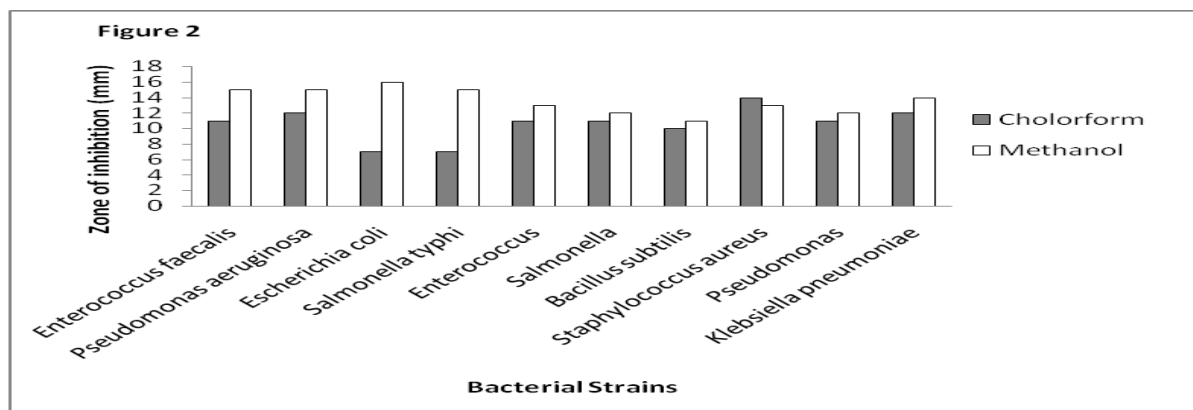


Figure 3: Zone of inhibition against different Solvent Extract of *Impatiens walleriana* by Disc diffusion method. Zone of inhibition showed by test sample with respect to activity: above 18: significant activity; 16-18 good activity; 13-15: low activity; 9-12: non-significant activity; below 8: no activity.

4. Discussion

There has been up till now, no research found on antimicrobial activity of *Impatiens walleriana*. To fill this extent, the present research was carried out. Flowers are venerated for their aroma and colors these sensual attributes are a profitable sign of the external features and the ornamental qualities as well as significant properties utilized for the culinary purpose. The much ornamental plant contains high levels of antioxidant compounds, which often even higher than common horticulture crops [14]. An essential parameter used to assess the antioxidant content is the determination of the total phenolic. Otakaret *al.*, demonstrated total phenolic content on *Impatiens walleriana* (pink, sweet flavor) gives 4.85g GAE/kg comparable lower to our results which give a higher amount of phenolic as 4.89-67.57g GAE /kg. Despite these values, nevertheless, these values were found higher from some horticultural and vegetables [15]. Anweret *al.*, demonstrated total antioxidant capacity utilizing DPPH on *Impatiens bicolor* plant evaluate values were of 92.2% and 62.6% which slightly higher than our present experimental results which are 80.4%, 70.7% and 52.5 respectively. Antibacterial activity of various solvent extract from *Impatiens balsamina* plant was screened out against gram positive and gram negative bacterial strain the result was found 16mm to 29mm in the range [16]. While, *Impatiens walleriana* extract were found effective as compare to *Impatiens blasamina* results was found up to 33 mm in a range difference in data might be due to numerous factors, for instance, these are two different species of *Balaseamcie* family, seasonal variation, and geographical location.

5. Conclusion

Future investigation is required to determine the sorts of other bioactive compounds in extracts of *Impatiens walleriana*. Because of their high antioxidant and antifungal activities, the *Impatiens walleriana* extracts have auspicious potential as natural antioxidants in the food industries, in the protection of foodstuffs against a range of food-related fungal species or in the pharmaceutical and cosmetic industries. In conclusion, the current research reveals that *Impatiens walleriana* local to Malaysia have substantial phytochemical and antioxidant activity.

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