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Research article

Untargeted MS-based small metabolite identification from the plant leaves and stems of *Impatiens balsamina*

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ABSTRACT

The identification of plant metabolites is very important for the understanding of plant physiology including plant growth, development and defense mechanism, particularly for herbal medicinal plants. The metabolite profile could possibly be used for future drug discovery since the pharmacological activities of the indigenous herbs have been proven for centuries. An untargeted mass spectrometric approach was used to identify metabolites from the leaves and stems of *Impatiens balsamina* using LC-DAD-MS/MS. The putative compounds are mostly from the groups of phenolic, organic and amino acids which are essential for plant growth and as intermediates for other compounds. Alanine appeared to be the main amino acid in the plant because many alanine derived metabolites were detected. There are also several secondary metabolites from the groups of benzopyrones, benzofuranones, naphthoquinones, alkaloids and flavonoids. The widely reported bioactive components such as kaempferol, quercetin and their glycosylated, lawsone and its derivatives were detected in this study. The results also revealed that aqueous methanol could extract flavonoids better than water, and mostly, flavonoids were detected from the leaf samples. The score plots of component analysis show that there is a minor variance in the metabolite profiles of water and aqueous methanolic extracts with 21.5 and 30.5% of the total variance for the first principal component at the positive and negative ion modes, respectively.
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1. Introduction

The genus of *Impatiens* consists of more than 1000 species of flowering plants from the family Balsaminaceae (Clifton, 2000). *Impatiens balsamina* is one of the species which is usually grown as an ornamental garden plant in Asia. This plant is also an annual herb that being used as indigenous medicine for the treatment of rheumatism, swelling, and fingernail inflammation since ancient time (Wang et al., 2011). The leaves and flowers of the plant are used to be crushed into mash and directly applied on the inflamed or burn skin. This plant is also widely used to treat warts and snakebite (Rajendran et al., 2014). The efficacy of this plant as a folk medicine has sparked the interest of researchers to investigate the bioactive chemicals from the plant in order to determine lead compounds for drug discovery.

With the current advancement in analytical technology, a broad range of phytochemicals such as fatty acids (Cahoon et al., 1999), naphthoquinones (Yang et al., 2001), coumarins (Panjchayupakaranant et al., 1995), phenolic acids (Bohm and Towers, 1962), flavonoids (Lei et al., 2010), anthocyanidins (Miles and Hagen, 1968) and saponins (Shoji et al., 1994) have been reported in this plant recently. Some of the compounds were found to contribute to the significant pharmacological activities. For instance, the derivatives of 1,4-naphthoquinones such as impatienol and balsaquinone had been proven to be highly selective cyclooxygenase-2 inhibitors which could be a new congener for novel nonsteroidal anti-inflammatory drug development (Oku and Ishiguro, 2002). Impatienol also exhibited significant testosterone 5α-reductase inhibitory activity to prevent the development of prostate cancer (Ishiguro et al., 2000). The saponin components of the plant extract were likely to be effective in reducing poison ivy dermatitis (Abrams Motz et al., 2012).

Extensive works have been carried out by worldwide researchers to identify the plant metabolites for further investigation. This includes the effort of the Metabolomics Standards Initiative

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which was endorsed by the Metabolomics Society of the United States of America in 2005, to refine the minimum set of reporting parameters (description of species and its genotype, growth history, specification of biochemical or physiological treatment, and harvest or postharvest condition) in an experiment (Fiehn et al., 2007a, 2007b). The reporting standards aim to facilitate experimental replication, comparisons of experimental data and designs, as well as between international publications for better understanding the metabolome of plants. The complex interaction between phytochemicals and biological systems explain the urge requirement of a powerful metabolomics based approach to investigate the metabolic pathway. It is believed that many new metabolites and their biological activities will be discovered from the plant extract in the near future.

In the present study, a high throughput liquid chromatography coupled to tandem mass spectrometer was used to identify small metabolites extracted by water, and aqueous methanol (50%v/v) from the leaves and stems of *I. balsamina*. This technique does not require excessive solvent consumption, time effective and high reliability for compound identification. The profile of small metabolites either in the leaves, stems or both parts of the plant is very important to investigate plant physiology and its significance. The detection of secondary metabolites could be used to understand the biological response of the plant. Secondary metabolites is the response of plants in their defense mechanisms to protect themselves against environmental stimuli and infection.

2. Materials and methods

2.1. Plants and chemicals

Impatiens balsamina L. (pink flower) was planted and harvested when the plant reached the flowering stage which was about after 10 weeks of planting. The plant tissues such as leaves and stems were separated and sun dried for 2 and 4 days, respectively. The picture of the plant is presented in Fig. 1. The species was authenticated and deposited in the Universiti Kebangsaan Malaysia Herbarium (Bangi, Selangor, Malaysia) with voucher specimen 40214.

Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (ferrozine, 97%), ferrous chloride (98%), potassium ferricyanide III (99%), potassium persulfate (≥99%), ferric chloride hexahydrate (97%), butylated hydroxyanisole (BHA, ≥98.5%) and trichloroacetic acid (≥99%) were sourced from Sigma–Aldrich (St. Louis, MO). Gallic acid (98%) and rutin (97%) were obtained from Acros Organics (Pittsburgh, PA). Sodium carbonate was bought from Merck (Darmstadt, Germany), whereas aluminium chloride was purchased from Fisher Scientific (Pittsburgh, PA). HPLC grade of methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Nanopure water (18.2 $M\Omega^{-cm}$) was produced from Barnstead NANOpure Diamond water purification system (Thermo, Waltham, MA).

2.2. Reflux extraction for plant samples

A 10 g sample of leaves and stems was extracted with 120 mL of 50% v/v methanol in a reflux extractor at 75 °C for 120 min. The extractions were carried out in triplicate. The crude extract solution was filtered and dried in *vacuo*. The extraction yield was 15.9 and 14.5% w/w for the leaves and stems, respectively. Consecutively, 1 mg/mL of the crude extract was reconstituted in methanol for LC-DAD-MS/MS analysis.

Fig. 1. Photo of *Impatien balsamina*.

2.3. Total phenolic and flavonoid content

The TPC of the extracts was analyzed by spectrophotometric method using Folin–Ciocalteu reagent. One mL of methanolic extract (1 mg/mL) was mixed with 50 μL of 50% Folin–Ciocalteu reagent and 2 mL of 2% sodium carbonate. The mixture was mixed thoroughly and incubated statically for 30 min at 30 °C. The absorbance of the solution was measured at 720 nm using a UV–Vis spectrophotometer (Perkin–Elmer Lambda 25, Waltham, MA). Gallic acid (0–1000 mg/L) was used as a standard for calibration curve preparation. The TPC was expressed as milligram of gallic acid equivalent (GAE) in a gram of dry weight plant extract. All assays were carried out in triplicate unless stated otherwise.

The TFC of the extracts was determined by spectrophotometric method using aluminium chloride. One mL of methanolic extract (1 mg/mL) was mixed with 1 mL of methanolic solution containing 2% aluminium chloride. A flavonoid-aluminium complex was formed after 15 min of incubation at 30 °C. The formation of the complex was measured at 430 nm using a UV–Vis spectrophotometer. Gallic acid (0–1000 mg/L) was used as a chemical standard for calibration curve preparation. The TFC was expressed as milligram of rutin equivalent (RE) in a gram of dry weight plant extract.

2.4. Antioxidant capacity of plant extracts

The radical scavenging capacity of the plant extracts was determined using radical DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Samples (1 mL) with different concentrations, ranging from 20 to 200 μg/mL were added to 2 mL of DPPH· solution (0.1 mM, 0.004%). The absorbance was measured at 520 nm after 30 min of

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