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Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*

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ABSTRACT

The sim fruit (*Rhodomyrtus tomentosa*) has long been used in folk medicine to treat diarrhoea, dysentery, and to boost the immune system. The purpose of this work was to determine its phenolic profile and to evaluate the changes of content during maturation, as well as the variations induced by environmental conditions. Using HPLC–ESI-HR-MS, 19 phenolic compounds (PCs) were tentatively characterised and included stilbenes and ellagitannins as major components, followed by anthocyanins, flavonols, and gallic acid. PCs were then further quantified by HPLC-DAD. Piceatannol, a promising health-promoting stilbene component, was the major PC in the fruit with a concentration of 2.3 mg/g dry weight at full maturity stage. This concentration is 1000–2000 times higher than that of red grapes, a major source of stilbene in the human diet. During maturation, the contents in piceatannol and other stilbenes, ellagitannins, and flavonols decreased while the anthocyanin content increased. Shade-grown sim fruits showed significantly higher piceatannol levels than sun-exposed fruits. Taken together, these findings highlight the potential of sim, an under-utilised plant species from South–East Asia, as a source of health-promoting fruits.

1. Introduction

Phenolic compounds (PCs) represent a large group of secondary metabolites produced in plants (Tsao, 2010). More than 8000 phenolic structures are currently known and they are generally classified into flavonoids (flavonols, flavones, flavanones, isoflavones, anthocyanins, and flavan-3-ols), phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid derivatives), phenolic alcohols, stilbenes and lignans (D'Archivio et al., 2007). Flavan-3-ols can be found as monomers (such as catechin and epicatechin) or polymers, also known as condensed tannins. Hydrolysable tannins are derivatives of gallic acid. The simplest hydrolysable tannins are gallotannins, which are simple polygalloyl esters of glucose. Oxidative coupling of galloyl groups converts gallotannins to the related ellagitannins (D'Archivio et al., 2007; Hagerman, 2010; Tsao, 2010).

Epidemiological studies strongly support a role for PCs in the prevention of cardiovascular diseases, cancers, osteoporosis, diabetes mellitus, arthritis and neurodegenerative diseases, which are associated to oxidative stress and chronic inflammation (Cicerale, Lucas, & Keast, 2012; Williamson & Manach, 2005). Although PCs have long been studied for their antioxidant properties, which are now well characterised *in vitro*, several studies have stressed that the mechanisms of biological actions of PCs extend beyond their antioxidant properties (Halliwell, Rafter, & Jenner, 2005). It is now believed that PCs may exert their beneficial action through the modulation of gene expression and the activity of a wide range of enzymes and cell receptors (D'Archivio, Filesi, Varì, Scazzocchio, & Masella, 2010; Tsao, 2010; Williamson & Manach, 2005). However, the biological actions of dietary PCs (including their bioavailability) strongly depend on their chemical structures (D'Archivio et al., 2010; Scalbert & Williamson, 2000). Determining the phenolic profile of a plant source is therefore of major importance in order to evaluate its impact on human health.

Sim (*Rhodomyrtus tomentosa* (Ait.) Hassk.) is a shrub of the Myrtaceae family, originating from South–East Asia. It is able to grow under various environmental conditions and has been

Abbreviations: PC, phenolic compound; LC, liquid chromatography; HR-MS, high-resolution mass spectrometry; ESI, electrospray ionisation; DAD, diode array detector; HHDP, hexahydroxydiphenyl; HD1, Hai Duong 1; HD2, Hai Duong 2; HB, Hoa Binh; TN1, Thai Nguyen 1; TN2, Thai Nguyen 2; PE, piceatannol equivalent; GAE, gallic acid equivalent; C3GE, cyanidin-3-glucoside equivalent; QE, quercetin equivalent; DW, dry weight; FW, fresh weight.

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reported as an invasive species in some areas of the globe where it was introduced as an ornamental plant. Parts of this plant (leaves, roots, buds and fruits) have been used in traditional Vietnamese, Chinese and Malay medicine for a long time. In particular, the fruits have been used to treat diarrhoea, dysentery, and to boost the immune system (Agro Forestry Tree Database, 1992; Do, 2011; Institute of Chinese Medicine, 2010). Sim fruits have an astringent taste and a deep purple colour at maturity. All these properties may, at least partially, be explained by the presence of PCs (tannins and anthocyanins). However, little information is available about the phenolic profile of these fruits. Only one report has recently described the presence of five anthocyanins, namely delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside in the sim fruit skin (Liu, Guo, & Sun, 2012), but nothing is known about the occurrence of other PCs.

Some studies have shown that maturity affects the accumulation and the profile of the PCs in fruits (Celli, Pereira-Netto, & Beta, 2011; Gruz, Ayaz, Torun, & Strnad, 2011; Shin, Ryu, Liu, Nock, & Watkins, 2008). The sim fruit displays a green colour that changes into a red or purple colour during the maturation process, suggesting a change in the phenolic profile. Furthermore, it is known that the environmental conditions such as light, soil type, and stress factors can also affect the phenolic content of the fruit (Guo, Han, & Wang, 2008; Shin et al., 2008; Wang, 2006; Wang, 2007; Wang, Chen, & Wang, 2009).

The main purpose of this work was to improve our knowledge on the phenolic composition of the *R. tomentosa* (Ait.) Hassk. fruit, in order to better understand its health-promoting properties. We have first focused on the identification of the main PCs in the sim fruit using high performance liquid chromatography (HPLC)/high-resolution mass spectrometry (HR-MS). Secondly, using HPLC coupled with a diode array detector (DAD), we studied the variations in the contents of the identified PCs according to the maturity stage and the growing location.

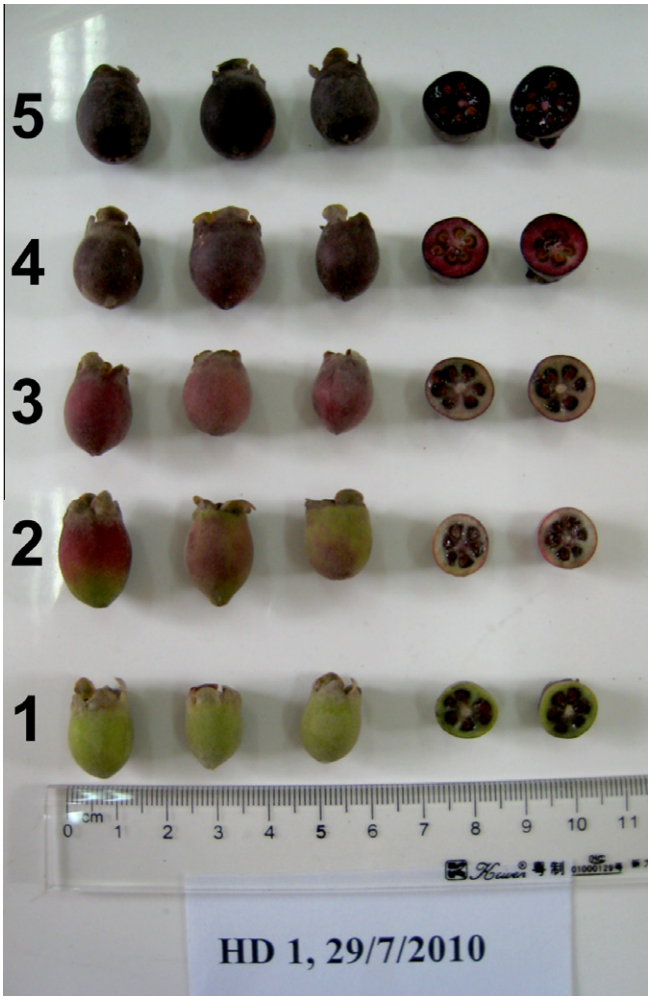

Fig. 1. Sim fruits (*Rhodomyrtus tomentosa*) at maturity stage 1–5.

2. Materials and methods

2.1. Sample collection

The sim fruits (*R. tomentosa* (Ait.) Hassk.) were harvested from five locations in the mountains of Hai Duong (HD1, HD2), Thai Nguyen (TN1, TN2) and Hoa Binh (HB) provinces in July and August 2010. The plants were identified at the species level as *R. tomentosa* (Ait.) Hassk by morphologic comparisons of leaves, buds, flowers, and fruits with the description of Pham (2000) and with the holotype (Code 05952) hold in the museum of the faculty of biology (Hanoi University of Science, Vietnam National University). Sim plants in HD1, TN1 and HB were exposed to sun light, while those in HD2 and TN2 were shade-grown. In each location, the fruits were hand-picked from three separate lots with about 30 plants per lot. The fruits were placed in a plastic box, kept on ice and transported to the laboratory on the same day. The fruits were then classified into five maturity stages: M1 (green with red streaks), M2 (half red), M3 (fully red), M4 (purple) and M5 (dark purple and softer) (Fig. 1). For each maturity stage and each location, approximately 1.5 kg of fruits was collected. A representative fruit sample was prepared by mixing 300 g of fruits at stage M4 from each location. The fruits were then frozen, freeze-dried, ground and stored at −53 °C under nitrogen until analysis.

2.2. Chemicals and reagents

Gallic acid, piceatannol, cyanidin-3-glucoside, ferulic acid, and quercetin dihydrate standards were purchased from Sigma–Aldrich (St. Louis, MO). Resveratrol was obtained from ExtraSynthese (Genay, France). Acetone of analytical grade was obtained from VWR-Prolabo (Briare, France). Methanol, acetonitrile, formic acid of HPLC grade were supplied by Biosolve (Valkenswaard, The Netherlands).

2.3. Sample preparation

In order to identify the main PCs, the representative sample was first extracted and fractionated. Approximately 0.4 g of powdered freeze-dried fruit was mixed with 8 ml of acetone: water: acetic acid (50:49:1; v/v/v) and shaken for one hour at 37 °C. After centrifugation at 3642*g* for 10 min at 4 °C, the supernatant was collected and the residue was extracted two more times with the same quantity of the same solvent. Supernatants from the three extraction steps were combined and evaporated to dryness by rotary evaporating at 40 °C. For fractionation, 360 mg Sep-Pak® Plus C18 cartridges (Waters, Milford, MA) were placed onto a vacuum manifold and conditioned with 3 ml of ethanol 100%, followed by 3 ml ethanol 50% and 3 ml water. The crude extract was first resuspended in 3 ml water and applied to the pre-conditioned cartridge. Stepwise elution was then performed by applying successive 3 ml aqueous solutions with increasing ethanol concentrations (0%; 5%; 7%; 10%; 15%; 20%; 25%; 50%; 75%, and 100%), and gave 10 fractions, which were collected into 15 ml tubes. Fractions were evaporated to dryness in a SpeedVac, resuspended in 1 ml methanol