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Contents of therapeutic metabolites in *Swertia chirayita* correlate with the expression profiles of multiple genes in corresponding biosynthesis pathways

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ABSTRACT

Swertia chirayita, an endangered medicinal herb, contains three major secondary metabolites swertiamarin, amarogentin and mangiferin, exhibiting valuable therapeutic traits. No information exists as of today on the biosynthesis of these metabolites in *S. chirayita*. The current study reports the expression profiling of swertiamarin, amarogentin and mangiferin biosynthesis pathway genes and their correlation with the respective metabolites content in different tissues of *S. chirayita*. Root tissues of greenhouse grown plants contained the maximum amount of secoiridoids (swertiamarin, 2.8% of fr. wt and amarogentin, 0.1% of fr. wt), whereas maximum accumulation of mangiferin (1.0% of fr. wt) was observed in floral organs. Differential gene expression analysis and their subsequent principal component analysis unveiled ten genes (encoding HMGR, PMK, MVK, ISPD, ISPE, GES, G10H, 8HGO, IS and 7DLGT) of the secoiridoids biosynthesis pathway and five genes (encoding EPSPS, PAL, ADT, CM and CS) of mangiferin biosynthesis with elevated transcript amounts in relation to corresponding metabolite contents. Three genes of the secoiridoids biosynthesis pathway (encoding PMK, ISPD and IS) showed elevated levels (~57–104 fold increase in roots), and *EPSPS* of mangiferin biosynthesis showed an about 117 fold increase in transcripts in leaf tissues of the greenhouse grown plants. The study does provide leads on potential candidate genes correlating with the metabolites biosynthesis in *S. chirayita* as an initiative towards its genetic improvement.

1. Introduction

Swertia chirayita (Roxb. ex Fleming) H. Karst. (Family: Gentianaceae), commonly known as 'Chirata', is a native of temperate Himalayas, found at an altitude of 1200–3000 m (4000–10,000 ft.) from Kashmir to Bhutan and in the Khasia range (4000–5000 ft.) (Joshi and Dhawan, 2005). Chirayita is known by an array of names such as Anaryatikta, Bhunimba, Chiratitka, Kairata in Sanskrit, Chiaravata in Urdu, Qasabuzzarirah in Arab and Farsi and Chirrato or Chiraita in Nepalese. It has been reported in various pharmaceutical indexes (Joshi and Dhawan, 2005) and in Eastern traditional systems of medicine such as Ayurveda, Unani and Siddha (Williamson, 2002), which gives a clear impression of the widespread use and pharmacological importance of this biennial herb.

The broad-spectrum biological activities of Chirata are attributed to the presence of a diverse array of pharmacologically important secondary metabolites belonging to different classes such as xanthones and their derivatives, flavonoids, terpenoids, iridoids

Abbreviations: 7-DLGT, 7-deoxyloganetic acid glucosyl transferase; 8-HGO, 8-hydroxygeraniol oxidoreductase; AAC, acetyl-CoA carboxylase; AACT, acetoacetyl-CoA thiolase; ADH, arogenate dehydrogenase; ADT, prephenate dehydratase; C3H, *p*-coumarate 3-hydroxylase; C4H, trans-cinnamate 4-hydroxylase; CM, chorismate mutase; CS, chorismate synthase; DAHPS, 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase; DHQD, 3-dehydroquinate dehydratase; DHQS, 3-dehydroquinate synthase; DL7H, 7-deoxyloganic acid hydroxylase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; G10H, geraniol 10-hydroxylase/8-oxidase; GDPS, geranyl diphosphate synthase; GES, geraniol synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; IO, iridoid oxidase; IPPI, isopentenyl diphosphate isomerase; IS, iridoid synthase; ISPD, 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase; ISPE, 4-(cytidine-5′-diphospho)-2-*C*-methyl-D-erythritol kinase; ISPF, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; ISPG, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase; ISPH, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase; LMT, loganic acid *O*-methyltransferase; MVDD, mevalonate diphosphate decarboxylase; MVK, mevalonate kinase; PAL, phenylalanine ammonia lyase; PAT, aspartate–prephenate aminotransferase; PHAT, phenylalanine (histidine) aminotransferase; PMK, phosphomevalonate kinase; SAK, shikimate kinase; SDH, shikimate dehydrogenase; SLS, secologanin synthase; TAL, tyrosine ammonia-lyase.

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and secoiridoid glycosides (Patil et al., 2013). The major phytochemicals of the bitter-tasting plant include swertiamarin, amarogentin (secoiridoid glucosides) and mangiferin, a xanthone C-glucoside (Phoboo et al., 2013). Swertiamarin is reported to be effective against hepatitis (Wang et al., 2011) and shown to exhibit anti-diabetic (Vaidya et al., 2013), anticancer (Kavimani and Manisenthlkumar, 2000) and anti-arthritic (Saravanan et al., 2014) activities. Amarogentin is known to be anti-diabetic (Phoboo et al., 2013), anticancer (Pal et al., 2012; Saha et al., 2006) and antileishmanial (Medda et al., 1999; Ray et al., 1996), whereas mangiferin has been tested for its anti-diabetic, anti-atherosclerotic (Pardo-Andreu et al., 2008), anticancer and anti-HIV (Guha et al., 1996) and antiparkinsonian (Kavitha et al., 2013) behaviors.

Overexploitation, due to an increased market demand along with other factors such as low seed viability (Badola and Pal, 2002) and narrow geographic occurrence (Bhat et al., 2013) has led to the endangered status of plant. The molecular understanding of biosynthesis routes leading to the production of major chemical constituents is lacking.

Swertiamarin and amarogentin take the classical MVA/MEP route of terpene biosynthesis followed by the secoiridoid pathway (Fig. 1). These secoiridoids originate from a common intermediate sweroside, which is biosynthesized from geranyl diphosphate (GPP), a condensation product of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Then they are transformed to the end products through a cascade of chemical conversions along with the formation of intermediates such as irido-dial/trial, deoxyloganic acid, loganic acid and secologanic acid (Miettinen et al., 2014; Wang et al., 2001). Swertiamarin is the hydroxylation product of sweroside, whereas amarogentin is the biphenylcarboxylic acid derivative of the same. The biphenylcarboxylic acid moiety was presumed to be biosynthesized from *m*-hydroxybenzoyl-CoA by a polyketide-type pathway from phenylalanine via cinnamic acid and benzoic acid as reported in *Swertia japonica* (Kuwajima et al., 1990). A retrobiosynthetic ^{13}C NMR study on *S. chirayita* also proved the formation of biphenylcarboxylic acid moiety from *m*-hydroxybenzoic acid, the later being formed preferentially from an early shikimate pathway intermediate, rather than from phenylalanine via cinnamic acid and benzoic acid (Wang et al., 2001). However, minor fractions of phenylalanine-derived metabolites might also contribute to amarogentin biosynthesis, which awaits an experimental proof. Mangiferin, on the other hand, follows a combined phenylpropanoid/acetate route (Fig. 2), where it is biosynthesized from phenylalanine through *p*-coumaric acid/cinamic acid and malonic acid along with benzophenone intermediates such as iriflophenone and maclurin as described in *Anemarrhena asphodeloides* (Fujita and Inoue, 1977).

Apart from a few studies as to expression analysis and functional characterization of geraniol 10-hydroxylase (G10H), a P450 in the CYP76B subfamily in *Swertia mussotii*, no comprehensive study has been done to associate the expression status of swertiamarin, amarogentin and mangiferin pathway genes with their biosynthesis in *S. chirayita*. We report for the first time the expression analysis of 24 genes of swertiamarin/amarogentin

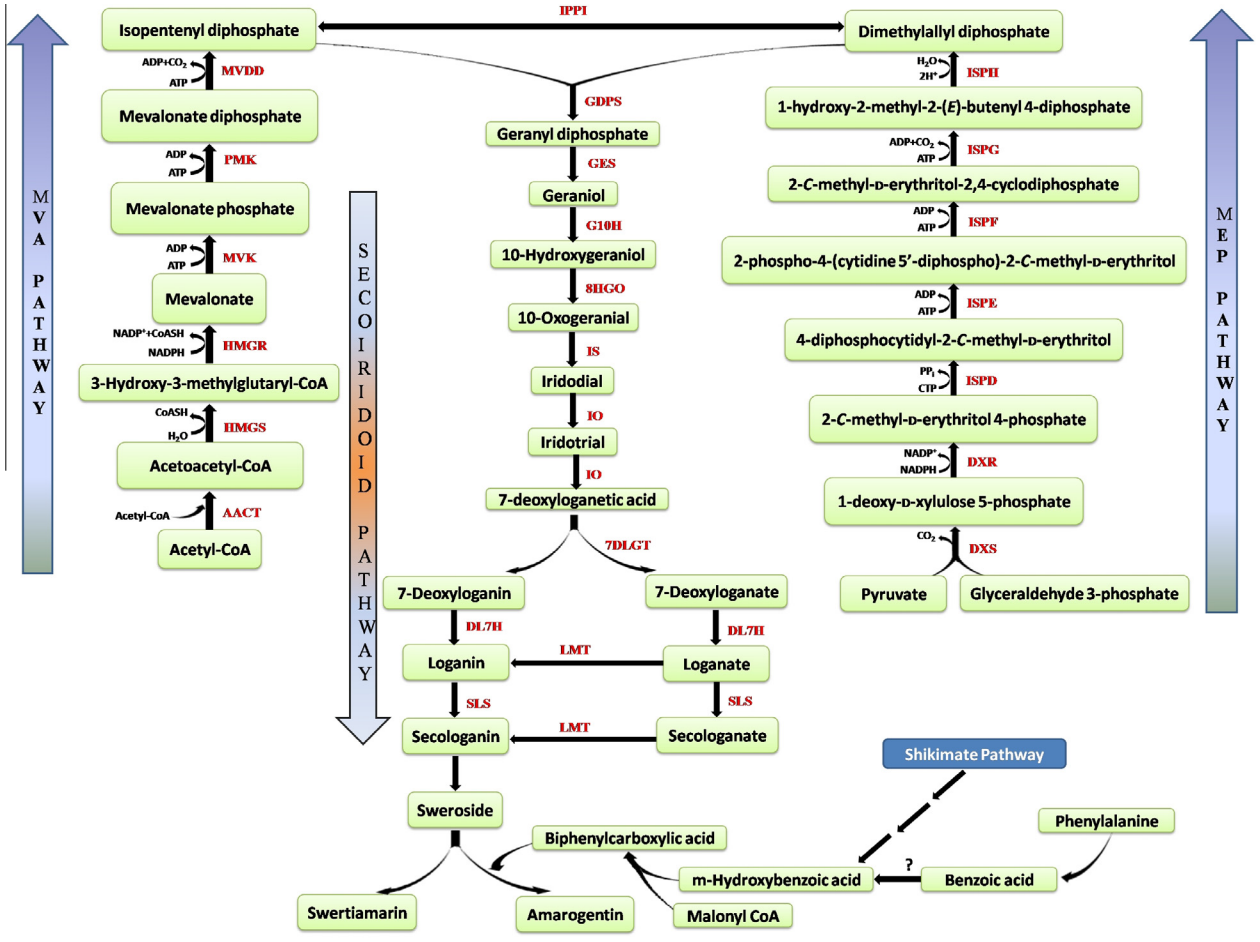

Fig. 1. Schematic representation of the proposed swertiamarin–amarogentin biosynthesis pathway (partly adapted from Miettinen et al. (2014)).

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