Phenolic characterisation of selected \textit{Salacia} species using LC-ESI-MS/MS analysis

Sulaiman C.T.\textsuperscript{a}, Thushar K.V.\textsuperscript{b}, Satheesh George\textsuperscript{c} and Indira Balachandran\textsuperscript{a}

\textsuperscript{a}Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, India; 
\textsuperscript{b}University of Calicut, Malappuram, India 
\textsuperscript{c}Department of Botany, St. Joseph’s College, Devagiri, Calicut, India

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Phenolic characterisation was carried out on the leaf of three \textit{Salacia} species such as \textit{Salacia chinensis}, \textit{Salacia fruticosa} and \textit{Salacia oblonga} using liquid chromatography coupled with quadrupole time of flight mass spectrometry equipped with electrospray ionisation interface. The estimation of total phenolics was carried out spectrophotometrically using Folin–Ciocalteu method. HPLC diode-array detection has been used for the preliminary identification of phenolic compounds, and liquid chromatography and mass spectrometry analyses were employed for their characterisation. The fragmentation patterns of the compounds during collision-induced dissociation led to the structural elucidation of the separated compounds.

\textbf{Keywords:} \textit{Salacia}; phenolics; LC/MS; MS/MS fragmentation

1. Introduction

\textit{Salacia} L. (Celastraceae) is a large genus of about 200 species distributed in tropical America, Africa and Asia. About 21 species occur in India (Singh et al. 2000). This genus, mainly consisting of climbing or creeping shrubs or rarely small trees, is known to elaborate anthocyanidines, catechins, phenolic acids, quinones, friedoleananes, quinonemethide and related triterpenoids (celastroloids), mangiferin, gutta-percha and dulcitol. \textit{Salacia fruticosa} is endemic to the Western Ghats and its distribution is restricted to the states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala.

\textit{Salacia} species have been used as anti-diabetic and anti-obese medicines for several thousand years in Ayurvedic medicinal literature. Increasing evidence suggests regulatory effects of this plant on exogenous nutrients, including $\alpha$-glucosidase inhibitor activity and pancreatic lipase inhibitor activity in the small intestine (Huanga et al. 2006).

\textit{Salacia oblonga} is a woody climbing plant found in the submontane forests of Sri Lanka and India. The roots and stems of this plant have been extensively used in the treatment of diabetes in the Ayurvedic system of Indian traditional medicine. (Wolfa & Weisbrode 2003).

The liquid chromatography coupled with quadrupole time of flight mass spectrometry equipped with electrospray ionisation interface has become the best method for separation, identification and characterisation of compounds from natural products. HPLC combined with PDA and mass analysers such as time of flight has been used for the sensitive and efficient metabolite characterisation (Wu et al. 2013). The combination of high-performance liquid chromatography and mass spectrometry (LC/MS) had a significant impact on drug development over the past decade. Continual improvements in LC/MS interface technologies combined with.
powerful features for structure analysis, qualitative and quantitative, have resulted in a widened scope of application (Sulaiman et al. 2012).

2. Results and discussion

2.1 Total phenolic content

The total phenolic content (TPC) was estimated spectrophotometrically (Supplementary Table 1.1). The phenolic contents were expressed as milligram gallic acid equivalent per gram of extract (mg GAE/g). The highest TPC was exhibited by S. oblonga (23.34 ± 0.14 mg GAE) followed by S. fruticosa (13.50 ± 0.12 mg GAE) and S. chinensis (6.72 ± 0.10 mg GAE). The result indicated that the leaf extracts of selected Salacia spp. are rich in phenolics. The evaluation of phenolics is an important concept of phytochemical studies as they are responsible for numerous biological and pharmacological properties such as hypoglycaemic, anti-inflammatory, anti-bacterial, anti-platelet aggregatory, anti-hypertensive, analgesic, anti-cancer and antiatherosclerotic due to their strong antioxidant and free radical-scavenging activities (Sulaiman et al. 2014).

2.2 HPLC analysis

The HPLC chromatogram of S. chinensis revealed major peaks at R_t 5.82, 6.66, 7.45 and 17.84 min. The online UV spectra of the separated compounds were also recorded. The compound with R_t 5.82 min revealed maximum absorption at 270 nm. The peak at 6.66 min indicated maximum absorption at 280 nm. The compound at R_t 17.84 min revealed maximum absorption at 230 and 278 nm. The UV λ_max indicated the phenolic nature of the separated compounds.

The diode-array detection chromatogram of S. fruticosa revealed only a few peaks with significant abundance. The peak at 2.09 min indicated maximum absorbance at 275 nm. The highest abundance was observed for the peak separated at 3.10 min which indicated maximum absorbance at 270 nm. The online UV spectra of the resolved peaks indicated that the same might be phenolics.

The HPLC chromatogram of S. oblonga revealed major peaks at 2.67, 2.86, 4.16, 4.86, 5.17 and 5.81 min. The peaks with R_t 2.67 and 4.16 min indicated maximum absorbance at 320 nm, peak at 2.86 indicated a maximum absorbance at 328 nm and the peak at 5.17 min indicated maximum absorbance at 260 and 328 nm.

2.3 LC–MS analysis

The analysis was performed by LC/MS in negative polarity mode. The mass fragmentation was performed by collision-induced dissociation (CID) in hexapole collision cell by varying the collision energy. The structural identification was carried out by comparing the mass fragments with the previously reported mass fragmentation patterns.

2.3.1 LC–MS/MS analysis

The total ion chromatograms obtained in negative polarity mode were extracted to obtain base peak chromatogram (BPC) (Supplementary Figures 1.4–1.6). The base peak ions and fragments obtained on CID are presented in Supplementary Table 1.3. The ion with m/z 191 [M − H]^- yielded a fragment with m/z 173 which was identified as quinic acid on the basis of mass fragmentation pattern (Rodriguez-Medina et al. 2009). It was found only in S. oblonga. The ion with m/z 169 [M − H]^- was found in all the three samples. On fragmentation, it yielded a
fragment with \( m/z \) 125 which was identified as gallic acid on the basis of mass fragmentation pattern. The loss of 44 u is due to the decarboxylation of acid group. The BPC indicated a molecular ion with \( m/z \) 193, which was fragmented with two major fragments at \( m/z \) 178 and 149 due to the loss of methyl group and \( \text{CO}_2 \) of ferulic acid (Gruz et al. 2008). The presence of ferulic acid was confirmed in both \( S. \) chinensis and \( S. \) fruticosa. The ion at \( m/z \) 179.03 indicated the deprotonated molecule \([M − \text{H}]^−\) of caffeic acid. The major fragment ions produced by MS/MS analysis were \( m/z \) 161.0 and \( m/z \) 135.0 corresponding to loss of water and carbon dioxide molecules, respectively, from the precursor ion. In general, deprotonated phenolic acids \([M − \text{H}]^−\) produce a typical fragmentation pattern after CID, characterised by the loss of a \( \text{CO}_2 \) (44 u) from the carboxylic acid group, providing an anion of \([M − \text{HCOO}]^−\) (Parejo et al. 2004).

The ion at \( m/z \) 305 presented a major fragment 225. The fragmentation pattern of the flavonoids was similar to those described previously and it was identified as gallic acid (Justesen 2000). The presence of gallic acid was also confirmed in all the three species of \( Salacia \). The presence of \( p \)-coumaryltartaric acid was identified in \( S. \) oblonga at \( m/z \) 295 as it presented two fragments at \( m/z \) 163 and 149. The fragment \( m/z \) 163 corresponded to the \( p \)-coumaric acid. The fragmentation pattern of coumaryltartaric acid has been reported previously (Rodriguez-Medina et al. 2009). The ion at \( m/z \) 463 was found only in \( S. \) oblonga. Upon CID, it yielded a fragment at \( m/z \) 301 which indicated that the compound was a quercetin derivative. The loss of hexosyl moiety (−162 u) confirmed the structure as quercetin hexoside.

The ion at \( m/z \) 353 was observed only for \( S. \) fruticosa and it yielded the base peak at \( m/z \) 191 (deprotonated quinic acid) and also yielded an ion at \( m/z \) 179 [caffeic acid − \( \text{H} \)] \(^−\). The compound was tentatively identified as caffeoylquinic acid. Similar fragmentation pattern was already reported by Clifford et al. (2003, 2005). The peak at \( m/z \) 198.17 yielded two major fragments with \( m/z \) 179 and 135 which was the fragmentation pattern of syringic acid, on losing a water molecule generating a major fragment ion at \( m/z \) 179 followed by a loss of carbon dioxide producing the other fragment at \( m/z \) 135. It was found to be present in all the species examined.

3. Conclusion

Application of HPLC–MS/MS technique provided useful information to characterise phenolic compounds in the leaf extracts of three \( Salacia \) species. The extraction of phenolics and their quantitative estimation are important analytical aspects owing to the strong evidence of biological activity of phenolics. The spectrophotometric estimations revealed that the leaves of all these three species are rich sources of phenolics. The HPLC-PDA chromatogram developed for each species can be used as chemical fingerprints for the proper identification of these species. Application of LC/MS technique in this study provided useful information to characterise phenolic compounds in the phenol-rich extracts of three \( Salacia \) species. Fragments produced during MS/MS CID analysis of the compounds reported earlier are the analytical features of these compounds which could be used to identify them in different extracts of other species as well.

**Supplementary material**

Experimental details are available online, alongside supplementary Figures 1.1–1.6 and Tables 1.1–1.2.

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