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Analytical Methods

Rapid characterisation and comparison of saponin profiles in the seeds of Korean *Leguminous* species using ultra performance liquid chromatography with photodiode array detector and electrospray ionisation/mass spectrometry (UPLC–PDA–ESI/MS) analysis

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ABSTRACT

The present work was reported on investigation of saponin profiles in nine different legume seeds, including soybean, adzuki bean, cowpea, common bean, scarlet runner bean, lentil, chick pea, hyacinth bean, and broad bean using ultra performance liquid chromatography with photodiode array detector and electrospray ionisation/mass spectrometry (UPLC–PDA–ESI/MS) technique. A total of twenty saponins were characterised under rapid and simple conditions within 15 min by the 80% methanol extracts of all species. Their chemical structures were elucidated as soyasaponin Ab (**1**), soyasaponin Ba (**2**), soyasaponin Bb (**3**), soyasaponin Bc (**4**), soyasaponin Bd (**5**), soyasaponin αg (**6**), soyasaponin βg (**7**), soyasaponin βa (**8**), soyasaponin γg (**9**), soyasaponin γa (**10**), azukisaponin VI (**11**), azukisaponin IV (**12**), azukisaponin II (**13**), AzII (**14**), AzIV (**15**), lablaboside E (**16**), lablaboside F (**17**), lablaboside D (**18**), chikusetusaponin IVa (**19**), and lablab saponin I (**20**). The individual and total saponin compositions exhibited remarkable differences in all legume seeds. In particular, soyasaponin βa (**8**) was detected the predominant composition in soybean, cowpea, and lentil with various concentrations. Interestingly, soybean, adzuki bean, common bean, and scarlet runner bean had high saponin contents, while chick pea and broad bean showed low contents.

1. Introduction

Legumes have been widely used in commercial and agricultural crops for decades from many countries (Iqbal, Khalil, Ateeq, & Khan, 2006). These species are excellent sources of many nutrients such as tocopherols, proteins, minerals, dietary fibers, carbohydrates, vitamins, and phytochemicals (Almeida Costa, Queiroz-Monici, Reis, & Oliveira, 2006; Amarowicz & Pegg, 2008; Boschin & Arnoldi, 2011). In recent years, legumes are increasingly of interest to food and medicinal industries due to beneficial properties for human health. For instance, European countries have the highest consumption, between 8 and 23 g/capita per day (Aguilera et al., 2009) and the consumption of legumes in USA has significantly increased because of functional and nutraceutical foods (Luthria & Pastor-Corrales, 2006). In addition, legumes are considered to be one of the most popular food sources in Korea for many years. The health beneficial activities of this species are attributed to the presence of various phytochemicals, namely, flavonoids, phenolic acids, tannins, triterpenic acid, and saponins (Amarowicz & Pegg, 2008; Kalogeropoulos et al., 2010; Shahidi & Naczk, 2004). For these reasons, many researchers have mainly focused on the secondary metabolites and their pharmacological effects from various legume species. Moreover, food and medicinal industries are increasingly interested in *Leguminous* crops with high phytochemical contents for the manufacture of supplements with therapeutic and preventive properties. In several phytochemicals, saponins are a diverse group of secondary metabolites with a structure constituting of steroid or triterpenoid aglycones and sugar moieties (Decroos et al., 2005; Hu, Lee, Hendrich, & Murphy, 2002; Kinjo et al., 1998). These compounds are widely distributed in crops, vegetables, fruits, and edible plants, and known to exhibit health beneficial effects on various illnesses. Numerous studies showed that saponins are of great interest in pharmaceutical aspects due to their biological effects, such as anticarcinogenic, anticancer, antifungal, anti-inflammatory, and antimicrobial

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activities (Gurfinkel & Rao, 2003; Konoshima, 1996; Nagata et al., 1985; Sparg, Light, & van Staden, 2004). Generally, it is well documented that analytical methods of saponins have been described in various food sources using HPLC–UV, HPLC/MS, HPLC–ELSD, ELISA, and HPTLC (Applebaum, Marco, & Birk, 1969; Chang, Han, Joh, & Kim, 2010; Guajardo-Flores, García-Patiño, Serna-Guerrero, Gutiérrez-Uribe, & Serna-Saldívar, 2012; Hu et al., 2002; Mbagwu, Okafor, & Ekeanyanwu, 2011; Price, Curl, & Fenwick, 1986). Unfortunately, these procedures have some inconvenience, namely, time-consuming, expensive apparatus, lack of reproducibility, and relative complicate. Also, there are few published researches on the simultaneous determination of the saponin compositions due to the low contents. Interestingly, the analytical time of most of the previous works was more than 30 min on the determination of saponins (Decroos et al., 2005; Hu et al., 2002; Kinjo et al., 1998). Although several scientists have focused on various foods and crops, including soybean, alfalfa, and ginseng (Chang et al., 2010; Guajardo-Flores et al., 2012; Lai et al., 2006), there is very limited information available on the saponin compositions of various *Leguminous* crops. Furthermore, little intelligence has been reported on the determination and comparison of saponin profiles on the basis of rapid and simple analysis. Up to data, UPLC analysis has the advantage of the high chromatographic peak capacity, fast analysis, and good sensitivity as well as resolution in comparison with other isolation methods (Guan, Lai, & Li, 2007). In addition, the UPLC coupled with mass spectrometry performs adequate structural information of multiple compounds (Han et al., 2011). Based on the above considerations, our work was designed to get available information regarding saponins in legume seeds using UPLC–PDA–ESI/MS analysis.

The purpose of the current research was the characterisation of saponin profiles in legume seeds. Moreover, this study was evaluated the comparison of saponin compositions with the application of the rapid and simple analytical method through UPLC coupled with mass technique from various Korean legume species.

2. Materials and methods

2.1. Plant materials and chemicals

Nine legume species, soybean (*Glycine max* (L.) Merr, Leguminosae), adzuki bean (*Vigna angularis* W. F. WIGHT, Leguminosae), cowpea (*Vigna unguiculata* (L.) Walp, Leguminosae), common bean (*Phaseolus vulgaris* (L.) Leguminosae), scarlet runner bean (*Phaseolus coccineus*, Leguminosae), lentil (*Lens culinaris* Medic, Leguminosae), chick pea (*Cicer arietimum*, Leguminosae), hyacinth bean (*Lablab purpureus*, Leguminosae), and broad bean (*Vicia fava*, Leguminosae) were grown in the experimental field of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Milyang, Gyeongnam, during 2010. After harvesting, legume seeds were washed with sterile water, and then air-dried for 3 days at 25 °C. The dried seeds were immediately freeze-dried and stored at −40 °C until analysis. Analytical grade methanol, water, and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Extraction of sample and UPLC conditions

The dried seeds of each legume were finely grounded for 3 min using a HR 2860 coffee grinder (Philips, Drachten, Netherlands). The pulverised seeds (60 mesh) were extracted with 10 ml of the 80% methanol at 25 °C for 24 h. The extracts of all samples were centrifuged at 3000*g* for 3 min, and filtered through a 0.45 μm syringe filter (Whtatman Inc., Maidstone, UK). Saponins in legume seeds were characterised by the Acquity™ UPLC system coupled on-line with a TQ (triple quadrupole) mass spectrometer detector (Waters, MA, USA) with an acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) at a flow rate of 0.4 ml/min. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient elution program was as follows: 0–1 min, 5% B; 2 min, 10% B; 10 min, 30% B; 12 min, 40% B; 15 min, 50% B; 20 min, 70% B; held at 100% B for 3 min, and then held for 3 min before returning to the initial conditions. The injection volume was 10 μl and the detection was recorded at 254 nm. The column temperature was set at 30 °C and the total running time was 20 min.

2.3. Mass spectrometry conditions

Mass spectra were scanned between *m*/*z* 400 and *m*/*z* 2000 in the ionisation mode (ESI^-) at a scan rate of 1.5 s/cycle and were monitored by a photo diode array (PDA) detector. The mass source temperature was maintained at 150 °C, and the desolvation temperature was set at 400 °C. The other mass spectrometric conditions were programmed as follows: capillary voltage 3.0 kV; cone voltage 60 V; collision energy 40 eV; desolvation gas flow of 850 l/h (N_2); cone gas flow of 50 l/h (N_2). Collision-induced fragmentation experiments were measured in the ion trap using helium as the collision gas.

3. Results and discussion

It is well demonstrated that solvents contribute to the highest efficiency of the extraction. Solvents with high polarity do afford good extraction results in comparison with those of low polarity (Liu, Ang, & Springer, 2000). In previously published data, ethanol and methanol were found to be the appropriate solvents regarding saponin (Guajardo-Flores et al., 2012; Iida et al., 1999). Moreover, the combination of ethanol and methanol with water offers a grateful effect for saponin extraction (Guajardo-Flores et al., 2012; Iida et al., 1999). In particular, the extraction conditions including 50–75% ethanol at high temperature or reflux and 50–80% methanol at room temperature have been widely used for saponins (Guajardo-Flores et al., 2012; Han et al., 2011). Although extraction temperature may cause an increase in the rate of phytochemical extraction, their contents tended to decrease due to the degradation of the molecule by extraction through high temperature (Harbourne, Marete, Christophe, Jacquier, & O'Riordan, 2013). Thus, we have chosen methanol solvent for saponin extraction at room temperature from various legume seeds. Also, the extraction solvent for saponins in the present work was measured by 80% methanol as supported by published data (Guajardo-Flores et al., 2012; Hu et al., 2002; Kinjo et al., 1998). It is well-known that the mass spectrometry is widely used as one of the most important analytical methods for determination of molecular weights regarding phytochemicals (Lee & Cho, 2012; Li et al., 2012). Specifically, the UPLC system coupled with mass spectrometry shows an excellent method for simultaneously identifying complicated compounds in edible sources due to the product ions produced from the fragmentation of a selected precursor ion (Dartora et al., 2011). Based on the above reasons, saponins in the seeds of nine different legume species were tentatively determined by UPLC–PDA–ESI/MS analysis. As presented in Fig. 1, a complete chromatographic separation of various saponins including major and minor peaks reached within 15 min at a wavelength of 254 nm. In other words, this method exhibited superior sensitivity and resolution as well as rapid separation. Table 1 shows retention times, molecular ions, and elementary compositions of the identified saponins by comparison with those