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Review

Extraction and quantification of saponins: A review

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ABSTRACT

Saponins, a second metabolites mainly derived from plant materials, have been used extensively in drug-related industry due to the pharmaceutical properties. These have driven the emergence of various new extraction technologies with the main purpose to optimize the yield in order to accommodate the recent need. The plants containing saponins are discussed, and their pharmaceutical properties and applications in food are highlighted. This review focuses on the saponin extraction with emphasis on conventional and green technology techniques employed in previous works by relating to their specific objective in each study. The quantification methods of saponins yield, i.e., spectrophotometric and chromatographic, are summarized and discussed. In addition, this review aims to provide a point of reference to researchers who wish to design experiment to suit their particular objective in swift.

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1. Introduction

Saponins are second metabolites which are widely distributed in the plant kingdom. It acts as a chemical barrier or shield in the plant defense system to counter pathogens and herbivores (Augustin, Kuzina, Anderson, & Bak, 2011). Therefore, it is found in plant tissues that are most vulnerable to fungal or bacterial attack or insect predation (Wina, Muetzel, & Becker, 2005). Saponins divided into two major classes which are triterpenoid and steroid glycosides which their structure characterization are varied by the numbers of sugar units attached at different positions (Hostettmann & Marston, 1995). The classification and occurrence of saponins in the plant kingdom are reviewed in detail by Vincken, Heng, de Groot, and Gruppen (2007).

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Saponins, which are derived from soapwort (*Saponaria officinalis* L.), have been widely used for centuries as household detergent (Sparg, Light, & van Staden, 2004) due to its amphiphilic nature with the presence of a lipid-soluble aglycone and water-soluble chain(s) in their structure (Güçlü-Üntündağ & Mazza, 2007). The seeds of *Barringtonia asiatica* Kurz (Lecythidaceae) which have known to contain saponins, have been used traditionally by native Asian and Pacific fisherman as fish poison to enhance their catches (Sparg et al., 2004). Saponin-containing plant materials, i.e., *Yucca schidigera*, alfafa, were used as feed additives to increase growth, milk or wool in ruminant production (Wina et al., 2005). The molluscicidal saponins derived from soapnut (*Sapindus mukorossi* Gaerth) have been found having inhibitory effects against golden apple snail, which is the major pests of rice and other aquatic crops in Asian countries (Huang, Liao, Kuo, Chang, & Wu, 2003).

The discovery of biological activities of saponins is not only limited to the traditional uses, but more recently, also in pharmaceutical applications (Güçlü-Üntündağ & Mazza, 2007; Sparg et al., 2004). Saponins have been found having pharmaceutical properties of hemolytic, molluscicidal, anti-inflammatory, antifungal or antiyeast, antibacterial or antimicrobial, antiparasitic, antitumor, and antiviral (Sparg et al., 2004). It employs as a starting point for the semi-synthesis of steroidal drugs in pharmaceutical industry. Sheng and Sun (2011) reviewed the clinical significance of triterpene saponins in prevention and treatment of metabolic and vascular disease.

The pharmaceutical property discoveries, especially anticancer, have intensified the seeking of saponins from plant materials. These have driven the emergence of various new extraction technologies with the main purpose of maximizing the yield in order to accommodate the recent need. Saponins are also known possessing mineral complexes of iron, zinc, and calcium (Milgate & Roberts, 1995). The beneficial effect of saponins intake in plasma cholesterol for human is another important factor that contributes to the continuous sorting of saponins (Milgate & Roberts, 1995). Besides anticancer (Cheng et al., 2011; Man, Gao, Zhang, Huang, & Liu, 2010; Waheed et al., 2012), saponins have been discovered scientifically having pharmaceutical properties of antioxidant (Chan, Khong, Iqbal, & Ismail, 2013; Dini, Tenore, & Dini, 2009; Li, Zu, et al., 2010), immunological adjuvant activities (Estrada, Katselis, Laarveld, & Barl, 2000; Sun, Chen, Wang, Wang, & Zhou, 2011; Verza et al., 2012), and hemolytic activities (Hassan et al., 2010; Sun et al., 2011).

Since saponins are currently the most interested subject of their potential for industrial processes and pharmacology, a correct selection of extraction technique through a review of appropriate literature is essential. Researchers from a variety of scientific backgrounds are often challenged by the initial extraction process prior to isolation and identification of specific saponins responsible for biological activities. The aim of this review is to summarize the selection of extraction methods from previous literature in respect to research focus in order to provide a quick reference in future experimental design.

2. Plant materials contain saponins

Saponins are mainly derived from various plant materials (Sparg et al., 2004; Vincken et al., 2007), but several of them are found in sea cucumber and starfish (Augustin et al., 2011; Demeyer et al., 2014). The most widely studied plant material that was found having saponins is ginseng (Kwon, Bélanger, Pare, & Yaylayan, 2003; Qian, Lu, Gao, & Li, 2009; Vongsangnak, Gua, Chauvatcharin, & Zhong, 2004; Wu, Lin, & Chau, 2001; Zhang & Cheng, 2006; Zhang, Liu, Qi, Li, & Wang, 2013), even though saponins derived from alfafa have been carried out as early by Van Atta, Guggolz, and Thompson (1961). Other plant materials which have been discovered containing saponins were soymilk (Lai, Hsieh, Huang, & Chou, 2013), sugar beet (Ridout, Price, Parkin, Dijoux, & Lavaud, 1994), soy and chickpea (Serventi et al., 2013), asparagus (Vázquez-Castilla et al., 2013), marion blackberry, strawberry, and plum fruit (Yoon & Wrolstad, 1984).

Saponin distribution has been found to vary in individual plant parts. For example, the roots of *Medicago truncatula* (Huhman, Berhow, & Sumner, 2005) and *Allium nigrum* L. (Mostafa et al., 2013) have been revealed containing the greatest total amount of saponins accumulation. The yam tuber cortex has been discovered to possess the highest amount of saponins of 582.53 μg/g dw which was about 2.55 times higher than tuber flesh of 227.86 μg/g dw (Lin & Yang, 2008). However, the total saponin concentration has been reported to contain the highest level in leaves from the four varieties of Swithchgrass (Lee et al., 2009) and greenhouse grown *Maesa lanceolata* (Theunis et al., 2007). Table 1 tabulates the saponins derived from different plant parts.

Since saponins fall in two categories which on water-soluble sugar units attached to a lipophilic steroid (C_{27}) or triterpenoid (C_{30}) moiety (Challinor & De Voss, 2013; Güçlü-Üntündağ & Mazza, 2007; Harborne & Baxter, 1999), therefore, the isolation and structure elucidation of triterpenoid (Connolly & Hill, 2010) and steroidal (Challinor & De Voss, 2013) saponins have been reviewed. Sparg et al. (2004) reviewed a list of plant species from which saponins have been isolated by categorizing them into triterpenoid and steroidal in period from 1998 to 2003. However, recent review on the triterpenoid and steroidal saponins derived from various plants is shown in Table 2. The elucidation and characterization of saponins structure are conducted on the basis of EI-MS (electrospray ionization-mass spectra), ^{1}H and ^{13}C NMR (nuclear magnetic resonance) data, such as in *Ipomoea batatas* (Dini et al., 2009), *Aralia taibaiensis* (Bi et al., 2012), and *Allium ampeloprasum* var. *porrum* L. (Adão, da Silva, & Parente, 2011).

3. Pharmaceutical properties of saponins

Saponins are rich in pharmaceutical properties and recently many studies focus on saponins' ability to increase immune responses (Estrada et al., 2000; Sun, 2006; Sun et al., 2011; Verza et al., 2012), and possession of antibacterial (Hassan et al., 2010; Iorizzi, Lanzotti, De Marino, Ranalli, & Zollo, 2002; Mostafa et al., 2013; Teshima et al., 2013), antioxidant (Bi et al., 2012; Chan et al., 2013; Dini et al., 2009; Li, Zu, et al., 2010; Lin, Yang, & Lin, 2011), anticancer (Cheng et al., 2011; Man, Gao, Zhang, Huang and Liu, 2010; Man, Gao, Zhang, Wang, et al., 2010), antidiabetic and anti-obesity properties (Joseph & Jini, 2013; Kimura, Ogawa, Katsube, Yokota, & Jisaka, 2008; Yun, 2010). Thus ginseng, which contains saponins, is included in most of the Chinese Medicinal Prescriptions, for example, Bianxia Xiexin decoction in treating gastroenteritis diseases (Wang et al., 2014). Seven structurally consecutive saponins derived from *Platycodon grandiflorum* have been discovered having hemolytic activities and adjuvant potentials on the immune responses to Newcastle disease virus-based combinant avian influenza vaccine in mice (Sun et al., 2011). Both Verza et al. (2012) and Sun (2006) revealed that saponin fractions derived from *Chenopodium quinoa* seeds and *Bupleurum chinense* enhanced hemolytic activities and adjuvant potentials on immune responses of mice against ovalbumin. Saponins obtained from *Polygala senega* L. were also suggested as potential vaccine adjuvants to increase specific immune responses (Estrada et al., 2000).

Hassan et al. (2010) reported that 100% methanol fraction of saponin-rich extracts from guar meal exhibited antibacterial activities against *Staphylococcus aureus*, *Salmonella Typhimurium* and *Escherichia coli*, however the results showed 20% and 60% methanol fractions stimulated *Lactobacillus* spp. growth. Aginoside saponins extracted from *A. nigrum* L. roots had significant antifungal activity (Mostafa et al., 2013). Saponins isolated from seeds of *Capsicum annum* L. showed higher antimicrobial activity against yeasts compared to common fungi (Iorizzi et al., 2002). The n-butanol extract of shallot basal plates and roots exhibited antifungal activity against plant pathogenic fungi (Teshima et al., 2013). Fruticoside I, a new steroidal saponins derived