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An Overview of Saponins – A Bioactive Group

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Abstract

Saponins are an important group of plant secondary metabolites that are widely distributed throughout the plant kingdom. These biomolecules can be divided into two main classes represented by triterpenoid and steroid glycosides whose structure varies according to the number of sugar units attached in different positions. Despite saponins have been historically considered as anti-nutritional factors, recent studies have indicated that some saponin stereoisomers may show a number of pharmacological activities, such as anti-tumor, antioxidative, anti-inflammatory, antidiabetic, and neuro-protective activities. Nevertheless, more attention in studying this group is necessary due to the fact that many active mechanisms are not fully elucidated. To have a systematic overview of saponin compounds, this review will describe the main aspects related to their structure, bioactivities and potential applications.

Keywords: bioactive compounds, saponins, secondary metabolites, steroid glycosides, triterpenoids

Introduction

Biologically active natural compounds have always played a significant role, whether their source is of plant or animal origin. Many scientific evidence show their benefits in reducing the risk of cancer, cardiovascular diseases, osteoporosis, inflammation, type II diabetes, and other chronic degenerative diseases, lowering the level of blood cholesterol and regulating the blood pressure, neutralizing the reactive oxygen species (Abuajah, 2017). Nowadays, when the concern about health of consumers is increasing, full comprehension is the only key to exploit effectively the potential benefits of bioactive compounds. In this sense, future investigations should focus towards optimizing the bioavailability of bioactive compounds

in order to enhance their *in vivo* chemoprotective and chemotherapeutic effects.

In the last decade, the saponins have been extensively reviewed by many research groups in order to elucidate their structure, distribution, biosynthesis, commercial and pharmacological importance as well as to find the most efficient extraction methods (Cheok et al., 2014; Singh and Chaudhuri, 2018; Aziz et al., 2019). Saponin is one of bioactive groups which is highly-appreciated and promising, due to its positive and various biological effects. Therefore, nowadays, the commercial applications of saponin doesn't resume to their use as a processing aid agent but also as a health supplement product. To have an overview and a systematic understanding of saponin compounds,

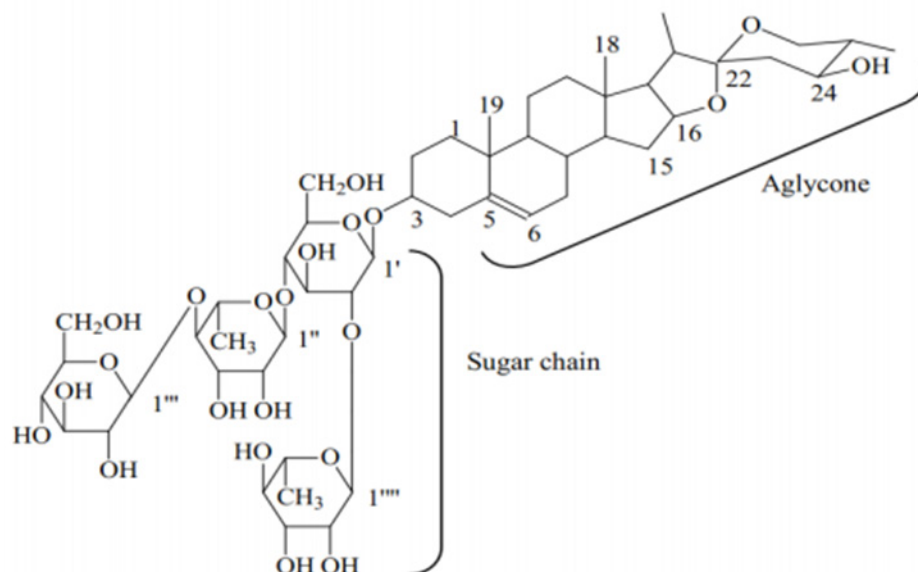


Figure 1. Structure of saponins (Moghimpour and Handali, 2015)

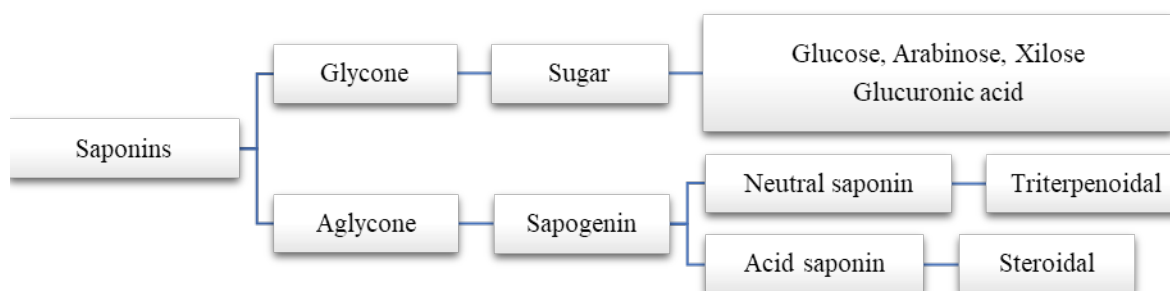


Figure 2. General classification of saponins

this review will describe the main aspects related to their structure, bioactivities and potential applications.

Chemical structure

Saponins is a class of high molecular weight amphiphilic compounds having triterpenoid and/or steroid aglycon as lipophilic moiety and sugars as hydrophilic moiety (Singh and Chaudhuri, 2018). Its large molecule has two parts: the sapogenin part and the sugar part (i.e aglycone and glycone), bound by a glycosidic bond (figure 1). The carbohydrate part might consist of several oligosaccharide chains as glucose, galactose, pentose, etc. Meanwhile, sapogenin part can be a steroid or triterpenoid.

Based on the different type of sapogenin part there are two classes of saponins. The steroidal saponins in which the sapogenin contains the characteristic four-ringed steroid nucleus, and a

typical extra furan and pyran heterocyclic rings. Due to the lack of these extra rings, the ginsenosides in ginseng are classified to the second class, the triterpenoid saponins even though they exhibit a steroidal structure. Meanwhile, the sapogenin of triterpenoid saponins has a five-ringed structure, which comes from the cyclization of the (3S)-2,3-epoxy-2,3-dihydrosqualene (Chaieb, 2010). Normally, one or more carbohydrate unit can bound with a sapogenin. If it is attached to the sapogenin through C₃, it is called monodesmosidic saponin. If saponin has more sugar moieties at C₂₆ or C₂₈, it is called bidesmosidic (Moghimpour and Handali, 2015).

Natural occurrence and properties

Distribution

A wide variety of crops and edible plants contains different compounds from the saponins

Table 1. Saponins content of various vegetable species (Savage, 2003; Güçlü-Üstündağ and Mazza, 2007; Tekeli, et al., 2007; Koomson et al., 2018; Zhang et al., 2018)

| Vegetable sources | Saponin content (g/kg of dry weight) |
|--|---|
| Chickpeas (<i>Cicer arietinum</i> L.) | 2.3 |
| Asparagus roots (<i>Asparagus officinalis</i>) | 15 |
| Peanut (<i>Arachis hypogaea</i>) | 16 |
| Green pea (<i>Pisum sativum</i>) | 1.8 |
| Eggplant (<i>Solanum melongena</i>) | 58 |
| Haricot bean (<i>Phaseolus vulgaris</i>) | 4.1 |
| Kidney beans (<i>Phaseolus vulgaris</i>) | 3.5 |
| Prickly nightshade (<i>Solanum torvum</i> Sw) | 8.6 |
| Lentils (<i>Lens culinaris</i> Medik.) | 1.1 |
| Spinach (<i>Spinacea oleracea</i>) | 15 |
| Oat (<i>Avena sativa</i>) | 1.3 |
| Mung bean (<i>Vigna radiata</i> L.) | 0.5 |
| Runner bean (<i>Phaseolus coccineus</i> L.) | 2.4 |
| Sugar beet leaves (<i>Beta vulgaris</i>) | 58 |
| Soya beans (<i>Glycine max</i> L.) | 6.5 |
| Yucca (<i>Yucca schidigera</i>) | 80 |
| Yellow split pea (<i>Pisum sativum</i>) | 1.1 |

class, their presence being usually identified in dicotyledonous and monocotyledonous plants. Triterpenoid saponins have been detected in many plants such as soybeans, beans, peas, tea, spinach, sugar beet, quinoa, citrus etc. Steroidal saponins are found in oats, tomato seed, asparagus, eggplant, etc. (Table 1). Cereals and grasses appear to be generally deficient in saponins, although there is still some exception, for example, oats, switchgrass, and kleingrass (De Geyter et al., 2007). The concentration of saponins found in barley husk were significantly higher (1.96 mg/g) than in wheat bran (1.16 mg/g). Saponins are more frequently found in the leaves and roots of plants. This may be the reason for the low concentration of saponins reported in barley husk and wheat bran. On the other hand, two different families of saponins synthesized in oats have been reported (steroids avenacosides and triterpenoid avenacin), found also in leaves and roots. It is important to note that limited research exists on the content of saponins in other cereals (Lopez-Perea et al., 2019).

Different type of saponins can be derived from different parts of plants and their distribution among the organs of plants varies considerably. Presently, commercial saponins are mainly extracted from two plant species: *Yucca Schidigera* and *Quillaja Saponaria*, which grow in the arid

areas of North and South America. There are also few marine species and certain insects containing saponins such as starfish, sponges and sea cucumbers.

Citrus fruits are one of the most important commercial fruit crops around the world. Many studies revealed that citrus waste represent a valuable source of bioactive compounds (phenolic compounds, carotenoids, sugars, fiber, volatile compounds, alkaloids, tannins, steroids, saponins, vitamins, minerals). It was reported that saponins present in citrus species were most likely to be the triterpenoids. From the point of view of the quantities, the highest level of saponin was reported in the leaves and peels of *C. sinensis* (0.98±0.03%) followed by *C. aurantifolia* (0.89±0.01%) and *C. paradisi* (0.87±0.06%) (Mathur et al., 2011; Ezeabara et al., 2014).

Like most of other bioactive groups, saponins are secondary metabolites. Although they are not essential for normal growth, development, or reproduction function of the organism, they play a role in the defense system, helping in protection, competition and other species interactions. Saponins are generally considered to have the ability to act against pathogens, pests and herbivores due to their antimicrobial, antifungal, antiparasitic, insecticidal and antifeedant properties (Chaieb, 2010). They are also released

by several plants (e.g *Medicago sativa* or alfalfa) to suppress the growth of other plants and prevent the competition for natural resources (Moses *et al.*, 2014). Research also found saponins present in defensive secretions of insects. For example, triterpenoid saponins were isolated from *Chrysomelidae*, especially from the *Platyphora* genus (Moghimpour and Handali, 2015).

Functional properties

Physical, chemical, and biological properties of saponins are the result of their structural complexity. These properties aren't generic, only a few of them are common to all members of this diverse group. However, these properties are the fundament of activities and applications of saponin.

Saponins are known to have a bitter, unpleasant taste and normally exists in colorless amorphous form. Each saponin has different solubility, depending on the solvent. For instance, saponins have high solubility in water, methanol, ethanol and other organic solvents, thus, these ones are the most commonly used for the saponins extraction. Variables such as temperature, composition and pH should also be considered as factors can influence the saponins solubility. Saponins have high melting point (generally above 200 °C) therefore, their stability to heat processing is remarkable, maintaining their biological activities also after the thermal treatment. Due to hydrothermal lysis or biological agents, the glycosidic bond (bond between the sugar chain and the aglycone), and those interglycosidides (bonds between the sugar residues) can be hydrolyzed in the presence of acids/alkali. The products of hydrolysis include aglycones, prosapogenins, sugar residues or monosaccharides depending on the hydrolysis method and conditions (Güçlü-Üstündağ and Mazza, 2007).

One significant property of saponins is acting as a surface-active agent like soap and detergent. Their name "saponin" is derived from Latin word "Sapo" meaning soap, due to this property. With one hydrophilic part (glycone) and one lipophilic part (sapogenin), in aqueous solution, saponins tend to align itself with the lipophilic part away from water, which leads to the reduction of the surface tension and cause the foaming. Similar to other surfactants, when the concentration of saponins is above the critical micelle concentration (CMC), they are able to form micelles in aqueous

solution, with the size and structure depending on the type of saponin. Consequently, saponin can enhance the solubility of other substances as well as other saponins. This capacity was observed through the increased solubility of progesterone and yellow OB in the presence of bidesmoside saponins from *Sapindus Mukurossi*. Saponins can also enhance cholesterol solubility, which is promising in hypocholesterolemia treatments (Mitra and Dungan, 2001). Compare to the synthetic surfactants, saponins are more effective in enhancing polycyclic aromatic hydrocarbons solubilization (Zhou *et al.*, 2011). The solubilizing effect of bisdesmosides on monodesmosides in water is well documented (Nakayama *et al.*, 1986).

Due to effects on cell membrane permeability, hemolytic activity of saponins is predominant among the various properties. By dissolving the blood cell membrane, it ruptures red blood cells and releases their cytoplasm into the bloodstream. This ability easily damages the red blood cells because they have no nucleus and therefore cannot affect membrane repair. However, not all saponins have hemolytic activity. While Guar meal and Quillaja saponin-rich extracts are hemolytic, other saponins from soybeans and yucca are not (Hassan *et al.*, 2009). Also, this activity may depend on solvents and extraction methods. A comparison was conducted between the saponins extraction from legumes by four protocols: EtOH/H₂O (1:1, v/v) for 2.5 h in H₂O bath at 95 C, pure MeOH in a Soxhlet apparatus for 50 h, H₂O for 5 h in a boiling H₂O bath, and phosphate-buffered saline at pH 7.3 while shaking for 2h. It was found that the EtOH/H₂O (1:1, v/v) extract showed the highest hemolytic activity (Khalil and El-Adawy, 1994). Experiment of Hassan *et al.* (2009) assessed the hemolytic activity of guar meal-saponin extracts by using reversed phase-high pressure liquid chromatography. The extract was separated into three peaks, eluting with 20%, 60% and 100% MeOH fractions. Results showed that the 20% and 60% MeOH fractions were not hemolytic at any concentration tested, while the 100% MeOH fraction was hemolytic until diluted (Hassan *et al.*, 2010).

Bioactivities & applications

Despite the variety in structure and properties, saponins and their derivatives have been reviewed having a number of biological activities, which

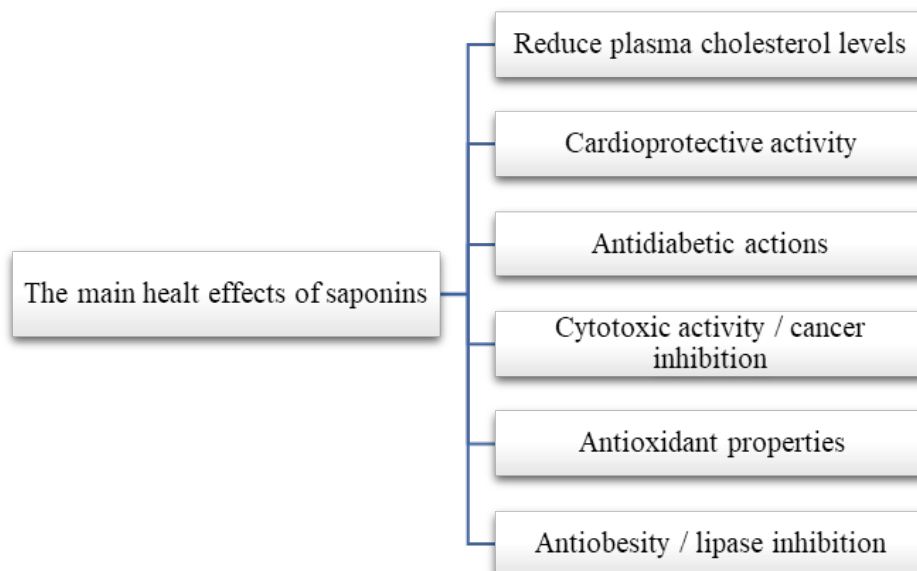


Figure 3. The main health effects of saponins

mostly positively affect human health (figure 3). Saponins have been historically considered as anti-nutritional factors, but contemporary researches have indicated that some saponins stereoisomers may show stereospecific pharmacological activities, such as anti-tumor; antioxidative, anti-photoaging, anti-inflammatory, antibacterial, antidiabetic, and neuro-protective activities, as well as stereoselective effects on ion channel current regulation, cardiovascular system, and immune system. Few significant activities can be mentioned such as adjuvant activity, cholesterol-binding activity, anti-inflammation and antitumor. Many types of research are focusing on their mechanism and applications on the development of dietary supplement products (Boo et al., 2015; Zhao 2016; El Barky et al., 2017; Saleri et al., 2017; Peng, 2018; Aziz et al., 2019).

Cardioprotective activity and hypocholesterolemic effect

Due to the structural similarities with cardioactive phytosterols along with important pharmacological effects such as hemolytic or permeabilization of cell membrane, serum cholesterol lowering and anticoagulant, saponins can be considered as having a positive influence on cardiovascular protection (Singh and Chaudhuri, 2018). The cholesterol-lowering activity of saponins has been well documented in animals and humans, different pathways being studied

and some elucidated. Saponins are recorded to have an effect in decreasing the absorption of cholesterol from intestine. A suggested mechanism is by forming a non-absorbable complex with cholesterol, the same mechanism as *in vitro*, which cannot pass through the intestinal wall. Besides, the fecal cholesterol excretion increases noticeably when saponins are included the daily diet, whereas the conversion of cholesterol into bile acid by the liver is significantly promoted (Zhao et al., 2008; Afrose et al., 2010).

Some phytosterols such as diosgenin and its derivatives are well recognized as cardioprotective agents that possess the ability to decrease the serum cholesterol in the intestinal tract by inhibiting cholesterol absorption (Genser et al., 2012). For example, the steroidal constituents such as dioscin, protodioscin, and trillin from the rhizomes of *Dioscorea nipponica* showed anti-hyperlipidemic activity by improving the levels of lipid peroxidation and SOD activity mediated through anti-lipase mechanism (Wang et al., 2012; Moghimipour et al., 2015).

Adjuvant activity

Saponins are used as adjuvants in oral vaccines formulation due to their stimulation of the responses of immune system against the pathogens. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed

forms of the microbe, its toxins, or one of its surface proteins. An adjuvant generally supports the immunogenicity of antigen, improving the efficacy of vaccine, thus minimizing the dose of antigen needed. Extracted saponins from *Quillaja Saponaria*, *Ginseng*, *Astragalus* are reviewed to have the ability to stimulate the cell-mediated immune system as well as enhancing the antibody production. Moreover, saponins have a prominent advantage as they are needed in low dose for adjuvant activity (Rajput *et al.*, 2007). The adjuvant ability of saponins has effects not only on the components of specific immunity but also on some non-specific immune reactions such as inflammation. The action mechanism is still unclear but several explanations have been proposed. It is believed that these compounds may induce the production of cytokines such as interleukins and interferons in animal system, which play an important role in immune cell differentiation and activation (Francis *et al.*, 2002).

Anticancer activity

High resistance of cancer cells constitutes a serious problem in chemotherapy and a challenging issue in the discovery of new cytotoxic treatments. Through researches, saponins have shown anticancer activity, which is regarded to be promising in applying in cancer therapy (Yildirim and Kutlu, 2015; Saleri *et al.*, 2017; Mbaveng, *et al.*, 2018). For all saponins, both aglycone and sugar structural features play an important role in determining cytotoxic activity (Podolak *et al.*, 2010). Shao *et al.* (1996) investigated the anticancer activity of asparagus crude saponins (ACS). Results indicated that the ACS inhibited the growth of human leukemia HL-60 cells at 75–100 µg/ml range. ACS concentrations greater than 200 µg/ml were cytotoxic to HL-60 cells. Man *et al.* (2009) have studied the ability of *Rhizoma Paradis saponins* (RPS) in treating pulmonary metastasis. The experiment conducted on mice showed that RPS has a powerful anti-proliferation effect by inducing apoptotic cell death and inhibition of the pulmonary metastasis by reducing expression of MMP-2 and MMP-9 and upregulating level of TIMP-2. Also, Mbaveng *et al.* 2018 evaluate the cytotoxicity and the modes of action of a naturally occurring oleanane-type triterpene saponin, isolated from the fruit of *Ardisia kivuensis* Taton, on a panel of 9 cancer cell lines. The studied oleanane-type triterpene saponin proved to

be a good cytotoxic molecule with potential in developing novel cytotoxic drugs to combat both sensitive and drug-resistant cancers. In another study, the investigated cytotoxic effect of triterpene saponins from the leaves of *Aralia elata* was investigated. The compounds showed significant cytotoxic activity against HL60 and A549 cancer cells (Zhang *et al.*, 2012).

Antibacterial and antifungal activities

The development of resistance to currently available antibiotics is a global concern, many studies reporting the existence of multidrug-resistant pathogens. In this sense, the use of natural antimicrobial compounds is important not only in food preservation, but also in the control of human and plant infectious diseases. Many researchers have studied the synergistic effect resulting from the association of antibiotics with saponins discovering new ways to treat infectious diseases. (Tamokou *et al.*, 2017; Tagousop *et al.*, 2018).

Citrus fruits contain various bioactive compounds in the peel, seeds and pulp that act as phytochemicals with bacterial growth-inhibiting, anti-fungal and anti-cancer activities. Saponins from citrus peel have been reported to have a wide range of pharmacological and medicinal properties. Moreover, the peel of citrus fruits is rich in flavonoid, glycosides, sitosterol, and volatile oils which can be efficiently used as drugs or as food supplements due to the fact that these compounds can act synergistically (Haroen *et al.*, 2018). Also, steroidal saponins have been reported to present antifungal properties against various plant pathogens, therefore they could be used as a green alternative for pathogens control in agriculture (Sadeghi *et al.*, 2013).

Antidiabetic activity

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels, which can damage the heart, blood vessels, eyes, kidneys, and nerves over time (Luyen *et al.*, 2018). In this sense, saponins have been shown to act through multiple mechanisms, including restoration of the insulin response, increase of plasma insulin levels, and induction of the release of insulin from the pancreas (Marrelli *et al.*, 2016).

Bioavailability, toxicity and safety of saponins

The major challenge in the use of saponins as medicines is related to their low bioavailability. It is considered that there are a number of factors that affect the bioavailability of saponins. One is related to the physicochemical properties of the drug itself (e.g., molecular weight, number of hydrogen bond donors and acceptors, solubility and chemical stability), and the other is the biological barriers that affect its entry to the systemic circulation (Gao et al., 2012). Of these, the high molecular weight and low membrane permeability are factors that significantly affect bioavailability, thus restricting the applicability of saponins as a drug candidate (Singh and Chaudhuri, 2018).

In general, saponins are evaluated as safe when at a normal orally intake, the majority of saponins being not toxic to humans. This is explained by their low absorption rate after ingestion, it cannot go into the bloodstream and if it does, the hemolytic activity will be weakened in the presence of plasma constituents. Typically, saponins will be hydrolyzed to sapogenins and sugars by the gut microflora. However, due to its hemolytic activity, saponins are toxic when directly injected to vein. The destruction of red blood cells can lead to reduced oxygen-carrying capacity of blood. Also, the delicate membranes of the glomerulus might be damaged due to massive production of red blood cells debris together (Savage, 2003).

Because of its oral safety to humans, saponin-containing extracts (such as quillaja extracts) are allowed to be used as food additives. In the USA, Quillaia, a commercial preparation of saponin from *Quillaia Saponaria*, is permitted by the United States Food and Drug Administration (FDA) for direct addition to products for human consumption. It is also approved as Generally Recognized as Safe (GRAS) designation by Flavor and Extract Manufacturers' Association (FEMA). Safety evaluation of quillaja extracts conducted by JEFCA took all relevant information on its toxicity and dietary exposure into consideration. At first, the committee allocated a temporary ADI of 0–5 mg/kg bw for the unpurified extract. Then, after reviewing new information relating to the chemical characterization of quillaia extracts, quillaja extracts were classified as type 1 ('unpurified') and type 2 ('semi-purified') based on their saponin

content, 20–26% and 75–90% respectively, the Acceptable Daily Intake (ADI) values being based on the saponin content of the extracts. However, at the present, the ADI is grouped to express for quillaia saponins as taking the lower end of the specified range, 0–1 mg/kg bw (FAO/WHO Expert Committee on Food Additives).

In term of toxicity, saponins are highly involved in plant defense mechanisms against plant diseases and pests. Steroidal saponins have been also reported to present antifungal properties with efficacy depended on the saponin type and the chemical structure and number sugar moiety. A typical example of the defensive role of saponins in vegetable crops is α -tomatine which provides protection to tomato plants against various pathogenic fungi and bacteria (Gonzalez-Lamothé et al., 2009; Teshima et al., 2013; Di Gioia and Petropoulos, 2019).

Although saponins are widely present in the plant kingdom, their toxicity to mammals is actually recorded only in a small number of plants and it depends on factors such as composition, source, concentration, etc. For example, the glycoside of mediagenic acid, which is present in alfalfa, is responsible for toxic effects on livestock and poultry (Liener, 2003).

Industrial applications

Various properties and activities of saponins are exploited in different industry sectors, including food, pharmaceutical, cosmetics or agricultural. Due to the surface activity, the extracts of saponins are widely utilized as emulsifiers, foam intensification agents and detergents. In term of non-food applications, saponins extracts are used in shampoo, liquid detergents, toothpaste, in addition to providing antimicrobial activity. Their level of detergent activity is light enough to be used on sensitive skin such as acne without causing a rebound increase in sebum production. Decoctions of soapwort have been used to wash and restore ancient fabrics in stately homes; modern soaps are too harsh and disintegrate the fabrics (Güçlü-Üstündağ and Mazza, 2007). *Quillaia* extracts have also been used in the production of films as wetting agents to obtain a uniform distribution of silver halide microcrystals dispersed in gelatin (Martin et al., 1999).

In food industry, as mentioned above, saponins are allowed to be used as food additive main-

ly for its foaming properties. Saponins in *Quillaja Saponaria* extracts are used as foaming agents in carbonated beverages and cosmetics, as emulsifiers in preparations containing lipophilic colors or flavors. Quillaja saponins can be used for the production of low-cholesterol dairy food products based on the ability of saponin micelles to form insoluble aggregates with cholesterol, which can be easily removed by filtration. Likewise, licorice saponin extracts are used as flavor modifiers in baked foods, chewing gum, beverages, candies, herbs, seasonings, plant dietary supplements as a flavoring agent only with specific limitations (Tamura *et al.*, 2012).

Saponins have also been applied in food processing as antimicrobial and anti-yeast agents. Tamura *et al.* (2012) highlighted the extract of *Yucca schidigera* is suitable to apply in food preservation. The extract has properties suitable for processing and it is safe for consumption. It is also tasteless and odorless, no influence on the taste of foods being recorded. It is readily soluble in water and stable on heating. Its shelf life extension ability was shown in sponge cake at the addition of 0.2% of yucca extract, which inhibits the growth of fungi and yeasts when the food product was stored at room for one week. The strawberry jam mixed with smaller concentration of yucca extract (0.02% and 0.04%) and stored at room temperature for one week showed no microbiological changes, while the control jam was contaminated by fungi.

Medicinal plants and their products with saponins have a huge potential to be used as functional food ingredients, nutraceuticals and in pharmaceutical products. Saponins also have pharmaceutical applications as raw materials for production of hormones, immunological adjuvants, and as drugs. Some of the individual saponins, notably saponin QS-21, showed low toxicity and could be potentially used as adjuvant in the preparation of human vaccines (Martin *et al.*, 1999).

Current techniques applied in the extraction and analysis of saponins

Due to their special structural arrangement, extraction and isolation of saponins represent a real challenge. Conventional methods have been explored as well as the recent, relatively greener, efficient, solvent economic, time-saving, newer methods of extraction. So far, extensive researches

have been performed on the identification and analysis of saponins.

Qualitative analysis and extraction method

Saponins can be isolated from plant materials by extraction with organic solvents, which are commonly used in extraction, for example, ethanol, acetone, diethyl ether, etc. The presence of saponins in crude extract can be detected by thin-layer chromatography (TLC) without proceeding to further purification steps. Tests based on saponins properties such as hemolytic activity or surface-active activity can also be used. However, these properties are not representative for the whole group. In comparison, TLC is faster and more definite identification. Then, depending on the order of solvents, we can obtain fractions contain water-soluble or fat-soluble saponins.

As mentioned before, conventional or green technologies can be used for saponins extraction. The conventional extraction is based on the solubility of solute from plant material into the solvent, being both solvent and time consuming. Among the classical extraction technique, maceration, Soxhlet, and reflux extraction were frequently used. The green technologies include microwave-assisted, ultrasound-assisted, supercritical fluid, and accelerated solvent extraction. Among the main advantages of the green extraction methods reduction of solvent used volume, reduction of extraction time and energy used can be mentioned together with the fact that they are environmentally friendly (Cheok *et al.*, 2014).

Quantitative analysis

The two main methods used for the quantification of plant saponins are spectrophotometric and chromatographic methods. Spectrophotometry allows identifying total saponins content while through the chromatography a specific saponin compound can be quantified. Spectrophotometric method is based on the light absorption ability of a chemical compound at a certain wavelength range. As it is simple, fast and inexpensive technique, spectrophotometry became a popular method in the quantification of total saponins. The most common used spectrophotometric methods for the total saponins quantification are the vanillin-sulfuric acid assay and the hemolytic assay. When using spectrophotometric methods, there are few factors that need to be considered such as the selection of standard, of extraction method, wavelength, conditions to allow color development,

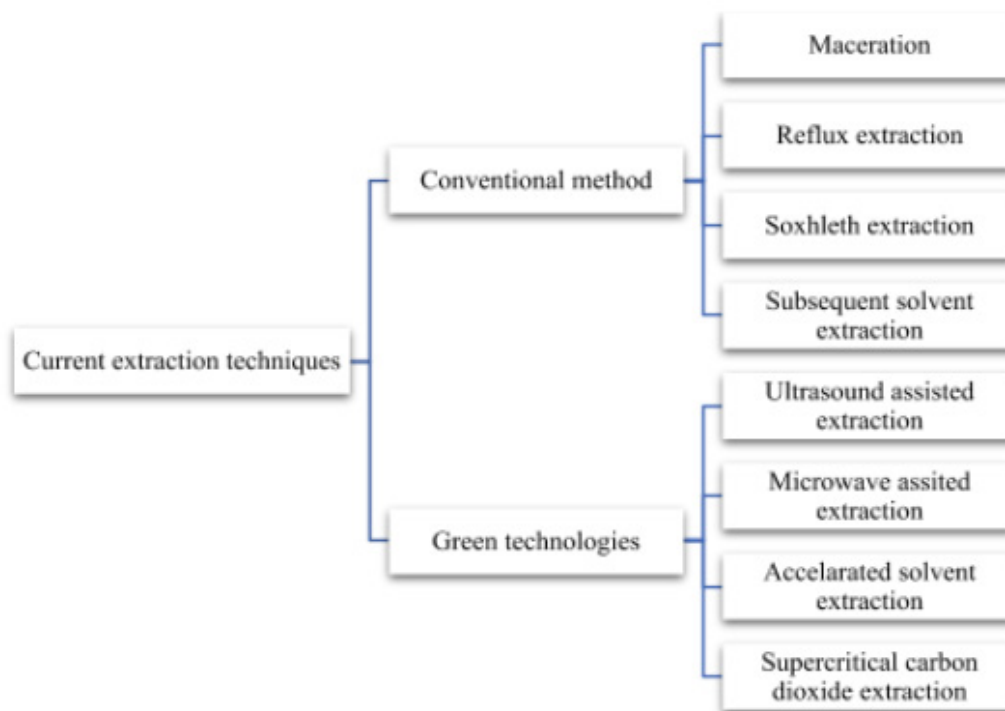


Figure 4 Current techniques applied in the extraction of saponins from plant materials

etc. The basic principle of spectrophotometric determination of saponins by vanillin-sulfuric acid assay is based on the reaction of oxidized triterpene saponins with vanillin. Usually, sulfuric acid is used as oxidant (but sometimes perchloric acid is also used) and the distinctive color of this reaction is purple. The selected wavelength is between 480–610 nm (based on the maximum absorption of formed purple color complex), the most used by the researchers being 544 nm (Cheek et al., 2014). The hemolytic assay is based on the reaction of saponins with blood reagent to release oxy-hemoglobin which color intensity can be measured spectrophotometrically.

The chromatographic separation is based on differential partitioning of saponins between the mobile and stationary phase. The various constituents of the mixture travel with different speeds due to their different affinities towards the stationary phase, causing them to separate. The method is suitable to identify and study a specific saponin compound. The most common chromatographic method for saponin analysis is high-performance liquid chromatography (Cheek et al., 2014). The isolation and characterization of specific saponin compound, together with its

quantification in a certain source plant offers not only a starting point and a reference for future researches but also valuable scientific reference for drug-related manufacturers who are interested to process a particular plant source further.

Conclusions

Saponins are a phytochemical group present in various plants. Their properties and bioactivities have positive effects on human health. Still, more research in studying this group of secondary metabolites is necessary view that many of saponin action mechanisms are not well understood. At the present, when the concern about consumers health is increasing, full comprehension is the only key to exploit effectively saponin potential benefits. In this sense, future investigations should focus towards optimizing the bioavailability of saponins in order to enhance their *in vivo* chemoprotective and chemotherapeutic effects.

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