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FOAMING GLYCOSIDES: A REVIEW

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Abstract—Foaming Glycosides, traditionally known for their detergent properties are generally responsible for expectorant action. Pentacyclic triterpenoid and steroidal types have different applications due to different linkages in their structures. Now-a-days their use is not just restricted to cleaning purposes but has widened to other fields too such as health, beverages and cosmeceuticals. Their availability in different plant genus and marine world help to meet up to the industrial demands. Due to the huge industrial demands scientists are looking out for the new formulations and improved ways for finding new extraction techniques for different classes of saponins. Wide applications of saponins have emerged during this last one decade and is considered to be a growing hub. The present review is an effort to consolidate the work done on this particular class of secondary metabolite.

Keywords—Pentacyclic triterpenoid, Secondary metabolite, Foaming glycosides, monodesmosidic

I. INTRODUCTION

Foaming Glycosides (Saponins) are low molecular weight secondary plant constituents containing either a tetracyclic steroidal or a pentacyclic triterpenoid aglycone with one or more sugar chains (1). A broader definition, which is not used in this opinion, would include also the steroidal alkaloid glycosides found in potatoes (2). Traditionally, they have been used as detergents, pesticides and molluscicides in addition to industrial applications as foaming and surface active agents (3, 4). They are generally identified by their bitter taste, throat irritation, forming foam in aqueous solutions, fish toxicity and ability to lyses erythrocytes. However, an example of exceptions is ginsenoside saponins which do not lyse erythrocytes. Some saponins even have been used as flavour enhancer and sweeteners in foods and cigarettes. For example, the flavour enhancer, licorice root extract is rich in the saponin glycyrrhizin and saponins from the roots of *Glycyrrhiza glabra* and leaves of *Abrus precatorius* (5) are 941-fold as sweet as sucrose and 60 times sweeter than cane sugar. Recent research has established saponins as the active components in many herbal medicines (6) and highlighted their contributions to the health benefits of foods such as soybeans (7, 8) and garlic (9). The commercial potential of saponins has resulted in the development of new processing strategies and re-evaluation of existing technologies (10) for their extraction and concentration (11).

The present review covers all the aspects of Saponins which have been researched in the last decade.

Chemical Nature of Foaming Glycosides

Foaming glycosides are synthesized by a common metabolic pathway starting from acetyl co-enzyme A, Mevalonic acid and then Squalene are the intermediary products for both triterpenoidal and steroidal saponins. In general, synthesis of cholesterol, other steroids, and saponins proceed through a common synthetic pathway. Saponins are glycosidic compounds (12, 13) constituting a fat-soluble nucleus called the aglycone that is a either triterpenoid (C-30) or neutral or alkaloid steroids (C-27) (14). One or more sugar side chains called glycones can be linked through ether and ester linkages to the aglycone nucleus at glycosylation sites. Triterpenoid saponins naturally occur as saponin or free aglycone forms, while steroid saponins occur only as saponins and never in the free aglycone form. The molecular weights of saponins range from 1000 to 1500 Daltons (15).

II. DISTRIBUTION-FOAMING GLYCOSIDES

The presence of saponins (Foaming glycosides) has been reported in more than 100 families of plants, in both wild and cultivated plants (16), in lower marine animals and in some bacteria, but are uncommon in higher animals. However, they can be found in a few marine sources such as starfish and sea cucumber (17).

The steroidal saponins are mainly found in monocotyledons (such as Agavaceae, Dioscoreaceae and Liliaceae), and triterpene saponins are predominantly present in dicotyledons (Leguminosae, Araliaceae, Caryophyllaceae) (18). While the main dietary sources of saponins are legumes (soybeans, chickpeas, mungbeans, peanuts, broad beans, kidney beans, lentils), they are also present in oats, allium species (leek, garlic), asparagus, tea, spinach, sugarbeet, and yam (19). Soap bark tree (*Quillaja saponaria*), fenugreek (*Trigonella foenum-graceum*), alfalfa (*Medicago sativa*), horse chestnut (*Aesculus hippocastanum*), licorice (Glycyrrhiza species such as *Glycyrrhiza glabra*), soapwort (*Saponaria officinalis*), Mojave yucca (*Yucca schidigera*), gypsophila genus (such as *Gypsophila paniculata*), sarsaparilla (*Smilax regelii* and other closely related species of *Smilax genus*) and ginseng (*Panax genus*) are the main non-food sources of saponins used in health and industrial applications.

Triterpenoid saponins are widely distributed in the plant kingdom and have been identified in over 500 plant species such as soybean, alfalfa, quillaja, peas, tea, spinach, sugar beet, quinoa, liquorices, sunflower, horse chestnut, ginseng, and guar (20). Steroid saponins occur predominantly in 85 species of the genera Agave, Discorea and Yucca and 56 other genera such as tomato, asparagus, ginseng, and oats. In legumes, saponins are associated with protein and therefore are concentrated in protein-rich fractions (21). Two major commercial sources of saponins are yucca (*Yucca schidigera*) and quillaja (*Quillaja saponaria*). Yucca is grown in the arid Mexican desert and southwestern USA, and quillaja is a tree grown in arid areas of Chile.

Types and numbers of saponin differ in their distribution among plants according to many factors such as the part, species and age of the plant. More than one kind of saponin may occur in the same species. Alfalfa saponins containing zanhic acid aglycone (trisdesmoside alfalfa saponin containing three sugar side chains attached to aglycone) and its γ-lacton (lucernic acid or glucuronic acid) are found in leaves but not in roots (22). Medicagenic acid aglycones saponin is found in the roots and is absent in the plant leaves and hederagnin saponins (monodesmoside alfalfa saponin containing one sugar side chain attached to aglycone) are identified in both roots and leaves of alfalfa.

Soybeans have at least four different saponins (23). The saponins in the mature soybean are divided into group A and group B soyasaponins on the basis of their aglycone structures. Group A soyasaponins are bisdesmosidic with alternate sugar compositions in both sets of oligosaccharides attached to the aglycone at the 3- and 21-hydroxyl positions. Group A saponins are found only in soybean hypocotyls, while group B saponins are widely distributed in legume seeds in both hypocotyls (germ) and cotyledons.

Foaming Glycosides- Structure and Concentrations

Foaming Glycosides (Saponins) are categorized according to the number of sugar chains in their structure as mono, di-, or tridesmosidic. Monodesmosidic saponins have a single sugar chain, normally attached at C-3. Bidesmosidic saponins have two sugar chains, often with one attached through an ether linkage at C-3 and one attached through an ester linkage at C-28 (triterpene saponins) or an ether linkage at C-26 (furastanol saponins). The most common monosaccharides include: D-glucose (Glc), D-galactose (Gal), D-glucuronic acid (GlcA), D-galacturonic acid (GalA), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), and D-fructose (Fuc). The nature of the aglycone and the functional groups on the aglycone backbone and number and nature of the sugars can vary greatly resulting in a very diverse group of compounds. Saponin concentrations differ among plants as a function of plant species, plant variety cuttings of the same plant part, degree of maturity, growing environment (sunlight intensity, rain, disease and insect attack, etc.), agronomic factors (climate and soil), cultivation year, location grown and season.

As a percentage of dry matter, fenugreek seed contains 5-6% while soybean seed (*Glycine max*) contains 0.5-6.5% (23), yucca contains 8-12% (24), quillaja contains 8-10%, alfalfa contains 0.5-9.5%, licorice root contains more than 3%, guar meal contains 5-13% saponin and the aerial part of *Medicago arborea* contains 1.9-3.4% saponins of dry matter (14).

Saponin concentration tends to be lower in the outer parts than the inner parts of the oat kernel, but is the converse in quinoa seeds. Alfalfa roots contain 2.41% while alfalfa leaves contain 1.53% of dry matter as saponin. Alfafla (*Medicago sativa* L.) roots and leaves contain saponins ranging from 2.6-3.8 and 0.3-2.4% of dry matter, respectively. *Quinoa* saponins are 0.9% and 2.3% of dry matter in the whole seed and the bran, respectively (25).

Saponin concentration of a plant during germination is higher than in a mature plant of the same species. For example, alfalfa saponin concentration in sprouts increases from 2mg/g at the beginning of germination (soya saponin) up to 6-8mg/g (0.6-0.8% of dry matter) at 8-16days of age. Although saponin contents increase with sprouting in some plants such as soybean, lucerne, mung beans, and peas, they decrease in others plants such as moth beans.

Planting season affects saponin content. Alfalfa saponin content is lower in spring and fall than content in midsummer. Zanhic acid saponin (aglycone is zanhic acid) is the highest late in the season when the level of medicagenic acid saponin (the precursor of zanhic acid) drops dramatically. Hederagenin saponins in alfalfa leaves ranged from 0.1-0.4% of the total aglycosides or 0.03mg/g of dry matter versus 0.82-1.32mg/g in alfalfa root. Also, concentrations of saponin types A, B and E of alfalfa leaves are 10-15.5, 15-30, and 1%, respectively.

Extraction procedure also plays a role in reported net saponin concentrations. For example, oat saponin content ranges between 0.011 and 0.029% of dry matter as determined by high pressure liquid chromatography (HPLC) (26). However, avenacoside A oat saponin was 0.04% of dry matter and avenacoside B was 1% of dry matter as determined by thin layer chromatography (TLC).

III. EXTRACTIONS

Due to the ability of certain saponins to facilitate the formation of foam/emulsions, care must be taken during extraction and pre-analytical extract purification steps to avoid this. Saponins are traditionally extracted into water/ethanol mixtures, after which the ethanol is removed by evaporation and the saponins extracted from the water phase into 1-butanol (27, 28). During the last decade there have been considerable efforts to improve this methodology, mainly on the extraction of ginseng saponins and glycyrrhizic acid, and also escin from horse chestnut. These extraction studies were recently reviewed by Güçlü-Üstündag and Mazza (29). Especially the use of supercritical CO_2 extraction in combination with modifiers such as methanol, ethanol or aqueous methanol has proven successful. Due to the presence of a lipid-soluble aglycone and water soluble sugar chain(s) in their structure, saponins are surface active compounds. In aqueous solutions, they form micelles as concentration reaches a critical level. Thereby, they have solubilisation properties for other compounds. However, due to the inherent relatively low solubility of many saponins, both in water and in a number of more lipophilic solvents, it may be difficult to keep them in solution for analysis, as the addition of other compounds (even other saponins) may enhance the solubility of the saponin(s) in question for analysis. Due to these problems, when doing analysis on saponins, one should be observant on whether the compounds are dissolved.

Chemical determination

High performance liquid chromatography (HPLC) is the method of choice for the separation of saponins (30, 31). Both normal phase and reverse phase columns have been used. However, reverse phase HPLC, mostly by the use of C18 columns and gradient elution, seems to be the preferred method. As most of the saponins do not possess chromophoric groups, either spectrophotometric detection with pre-column derivatisation with e.g. benzoyl chloride has been used (30) or detection with electrospray ionization mass spectrometry is possible (32, 27).

As an alternative to chromatographic analysis described above, enzyme-linked immunosorbent assays (ELISA) have been applied to extracts of ginseng derived drugs in a study comparing the ELISA method with the HPLC analysis (33). Also many other studies are available in recent literature, mostly dealing with ginsenosides as above or other saponins of medicinal interest such as saiko saponins (34). A bottleneck for the development and use of modern validated methods for saponins, and Madhuca saponins in particular, is the unavailability of pure reference standards. However, for a large number of saponins essential data for the ease of compound identification – both during purification from plant sources and during analysis - such as nuclear magnetic resonance (NMR) chemical shifts -, have been collectively published (35).

Biological Activities of Saponins

Saponins have been variously attributed with a diverse range of properties, some of which include both beneficial and detrimental effects on human health, pesticidal, insecticidal and molluscicidal activity, allelopathic action, antinutritional effects, sweetness and bitterness, and as phyto-protectants that defend plants against attack by microbes and herbivores (24).

Cell membrane permeability

They have a specific ability to form pores in membranes. Saponins have a lytic action on erythrocyte membranes. The hemolytic action is believed to be the result of the affinity of the aglycone moiety for the phospholipids present in the cell membrane with which they form insoluble complexes (36). The amount of glycosides required for permeabilisation is much lower for cholesterol-rich lipid layers than cholesterol-free membranes.

Hypoglycemic activity

The saponins present in fenugreek are responsible for hypoglycemic activity either by stimulating the cells or by suppressing the transfer of glucose from the stomach to the small intestine and the inhibition of glucose transport across the brush border of the small intestine.

Cholesterol metabolism

The saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects. Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion when saponin-rich foods such as soyabean, lucerne and chickpea are consumed. The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to go down.

Anti-inflammatory activity

The significant ameliorative activity of the saponins may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin along with its antioxidant property which inhibits the formation of ROS which also plays a major role in inflammation (37, 38).

Hypolipidaemic activity

The mechanism involved in the hypolipidemic activity is, saponins and high fiber content in the different plant extracts. The fiber significantly binds to cholesterol hence aiding its excretion. Saponins have also been shown to posses high degree of hypolipidaemic activity. The combine activity of saponins and fiber content of the plant extract brings about the reduction in plasma concentration of cholesterol and the lipids. Thus reducing the possible occurrence of coronary heart disease such as atherosclerosis (39).

Protein digestion

Saponins reduce protein digestibility probably by the formation of sparingly digestible saponin-protein complexes. Endogenous saponins affected the chymotrypsic hydrolysis of soyabean protein, particularly glycinin. The heat stability of bovine serum albumin was increased by the addition of soyasaponin due to electrostatic and hydrophobic interactions. The digestibility of the bovine serum albumin- soyasaponin complex is much lower than that of free bovine serum albumin indicating that complexing with saponin had an obstructing effect.

Antifungal activity

This activity of the steroidal saponin is associated with their aglycone moieties and the number and structure of monosaccharide units in their sugar chains (40).

Antimicrobial activity

Saponins show the antimicrobial activity by inhibiting the growth of Gram +ve or Gram -ve microorganism. Some saponins are not effective against Gram -ve microorganisms because of the reason that they are not able to penetrate into the cell membranes of the microorganisms (41, 42).

Other Biological activities

The saponins are also responsible for lowering cancer risks by lowering the blood cholesterol levels. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have shown to have an inverse relationship with the incidence of renal stones. They are also responsible for many other important activities Molluscidal, Anthelmintic, Antiulcerogenic, Anticancer, Antioxidant, Immunomodulatory, Anti-malarial, Anti-bacterial, Eczema, Analgesic, Anti-nociceptive, hepatoprotective (21, 43).

Toxicity

Many foaming glycosides exhibit toxic effects at high doses over long periods of time causing problems such as excessive salivation, vomiting, diarrhea, loss of appetite and manifestations of paralysis. Oral toxicity of saponins in warm-blooded animals is relatively low and LD50 (lethal dose, 50%) values are in the range 50-1000mg/kg However, they are highly toxic when given intravenously. The toxic effects of many saponins are neutralized by saliva of animals such as sheep (45) intestinal bacteria (44) and rumen bacteria (46). Cooking or heat processing can also detoxify saponins. For example, cooking decreases saponin content by 7-17% in chick peas, 40% in faba bean seeds and 72% in quinoa. Not all saponins are degraded at the same conditions and temperature. For example, oat saponins are not affected until heated to 140^0C for 3h. Degradation increases as the pH decreases from 7 to 4 in avenacoside A.

Saponin degradation sometimes induces activity of enzymes such as β-glycosidase that occur naturally in oat leaves. Removing the C-26-bound glucose moiety results in forming a monodesmosidic saponin with the highest antifungal activity. Also, avenacosides saponin A and B in oats are activated by the plant's enzymes in response to tissue damage or pathogen attack by fungi Saponins can also be hydrolyzed by cleaving the ester-linked sugars to yield carbohydrates and aglycones (31).

IV. CONCLUSION

Studies have revealed that Foaming glycosides have immense medicinal importance and further research can be carried out on the derivatives in which initial observation have shown anti-mutagenic and anti-inflammatory activity.

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