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## Saponin-Based, Biological-Active Surfactants from Plants

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/68062>

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### Abstract

Plants have the ability to synthesize almost unlimited number of substances. In many cases, these chemicals serve in plant defense mechanisms against microorganisms, insects, and herbivores. Generally, any part of the plant may contain the various active ingredients. Among the plant, active compounds are saponins, which are traditionally used as natural detergents. The name 'saponin' comes from the Latin word 'sapo,' which means 'soap' as saponins show the unique properties of foaming and emulsifying agents. Steroidal and triterpenoid saponins can be used in many industrial applications, from the preparation of steroid hormones in the pharmaceutical industry to utilization as food additives that exploit their non-ionic surfactant properties. Saponins also exhibit different biological activities. This chapter has been prepared by participants of the Marie Skłodowska-Curie Action—Research and Innovation Staff Exchange (RISE) in the framework of the proposal 'ECOSAPONIN.' Interactions between the participants, including chemists, physicists, technologists, microbiologists and botanists from four countries, will contribute to the development of collaborative ties and further promote research and development in the area of saponins in Europe and China. Although this chapter cannot provide a comprehensive account of the state of knowledge regarding plant saponins, we hope that it will help make saponins the focus of ongoing international cooperation.

**Keywords:** plants, saponins, surfactants, emulsifiers, biological activity

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## 1. Introduction

An increasing trend in the food, pharmaceutical, and cosmetic industry is the utilization of natural plant extracts or plant-derived compounds, as an alternative to the application of chemical or synthetic antimicrobials to combat spoilage microflora and pathogens [1, 2]. Furthermore, the nontoxic nature of chemicals in plants, positive healthy properties, consumer perception and acceptance of their use has been well demonstrated [3, 4].

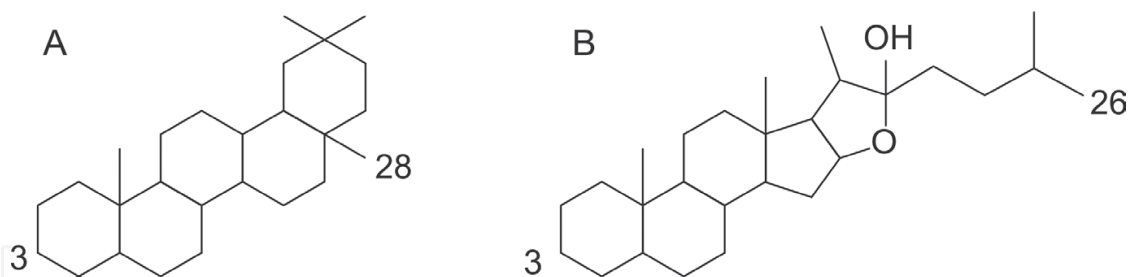
There are estimated 250,000–500,000 species of plants on Earth. A relatively small percentage (1–10%) of these is consumed as food by both humans and animal species. It is possible that a greater number are used for medicinal purposes. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants. Currently, antimicrobial plant extracts are of especial interest to chemists and microbiologists due to growing public awareness of the negative effects of the over-use of antibiotics and disinfectants [5].

Plants have the ability to synthesize an almost limitless array of substances. In many cases, these chemicals serve as plant-defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their flavors; others—quinones and tannins are responsible for plant pigmentation. Any part of the plant may contain active components. For instance, roots of ginseng plants contain active saponins and essential oils, while eucalyptus leaves are harvested for their essential oils and tannins. Some trees contain useful substances in their bark, leaves, and shoots [6]. Some of the same herbs and spices used by humans to season food can yield useful medicinal compounds. Among different compounds derived from plants, saponins deserve a special mention. These chemicals may be considered as a part of plants' defense systems. They have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants' [7].

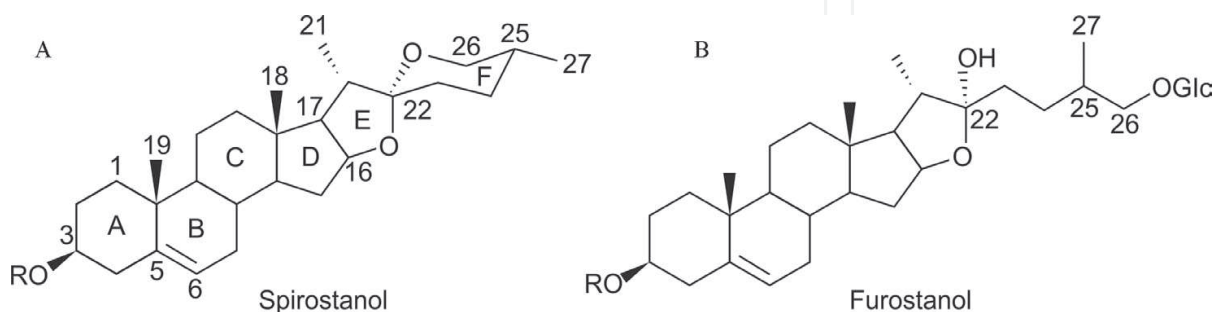
The physiochemical and biological properties of saponins have led to a number of traditional and industrial applications. They have traditionally been used as natural detergents. The combination of a hydrophobic aglycone backbone and hydrophilic sugar molecules confers foaming and emulsifying properties of saponins [8]. The name 'saponin' is derived from the Latin word 'sapo,' meaning soap, as a soapy lather forms when plants containing saponins are agitated in water. They also exhibit a variety of biological activities. Plant-derived triterpenoid and steroidal saponins have been used in the production of steroid hormones in the pharmaceutical industry, as food additives, fire extinguishers and in other industrial applications. Other interesting biological applications include their use in anti-inflammatory, hypocholesterolemic and immune-stimulating remedies [9, 10].

## 2. Molecular characteristics

Saponins are a class of substances with a rigid skeleton of at least four hydrocarbon rings to which sugars in groups of one or two are attached (usually not more than 10 units). Traditionally, they are subdivided into triterpenoid and steroid glycosides. Steroidal saponins are mainly compounds containing 27 carbon atoms forming the core structures: spirostan ( $16\beta,22:22\alpha,26$ -diepoxy-cholestan) and furostan ( $16\beta,22$ -epoxycholestan) [11–13] (**Figures 1 and 2**).



**Figure 1.** Structures of (A) triterpenoid and (B) steroidal saponins [8].



**Figure 2.** Structures of (A) spirostanol and (B) furostanol saponins [14].

There are 11 main classes of saponins: dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids. The oleanane skeleton is the most common, present in most orders of the Plant Kingdom [15, 16].

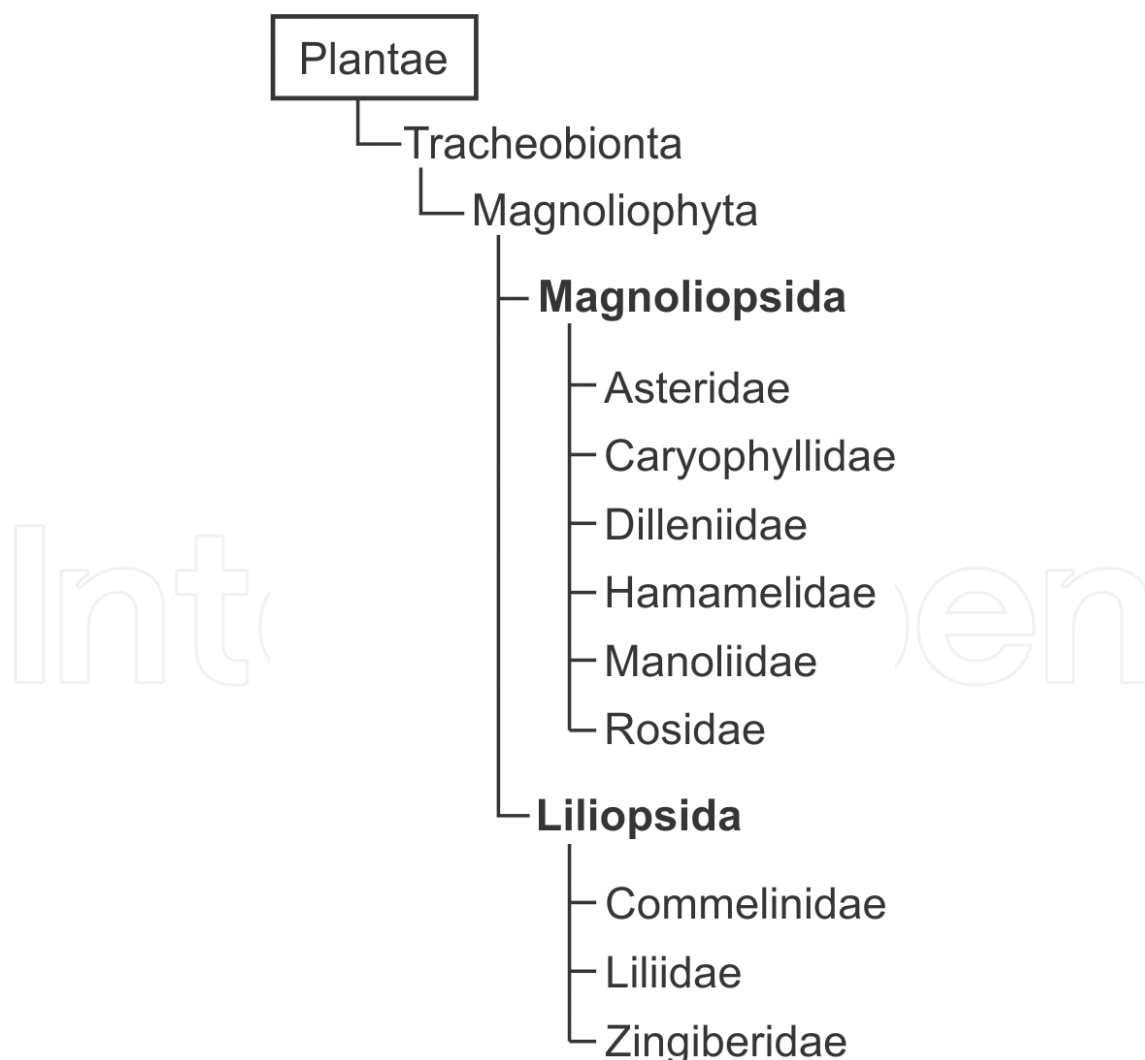
Saponins with the carbohydrate or oligosaccharide groups attached at the C-3 position are monodesmosidic, while saponins with carbohydrates attached at both the C-3 and C-26 or C-28 positions are bidesmosidic. The variety of a glycones, carbohydrates, and different attachment positions result in numerous types of saponins. The carbohydrate chains of saponins usually include: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, D-apiose, D-fucose, and D-glucuronic acid. The steroidal saponins usually show furostanol or spirostanol form. Additionally, both steroidal and triterpene saponins may contain other functional groups:  $-\text{OH}$ ,  $-\text{COOH}$ ,  $-\text{CH}_3$  that give them additional diversity [17].

The chemical structure of saponins may be transformed during storage or processing. The linkages between the sugar chain and the aglycones as well as between the sugar residues can undergo hydrolysis during acid or base treatment, hydrothermolysis or enzymatic/microbial transformations, resulting in the formation of aglycones, prosapogenins (partially hydrolyzed saponins), and sugar residues [17]. Therefore, the selection of methods appropriate to storage of plant material is a key part of each efficient technology [18–20].

### 3. Plant sources

The presence of saponins has been reported in more than 100 families of plants and in a few marine sources such as star fish and sea cucumber. Triterpene saponins are present in many

taxonomic plant groups. In particular, they can be found in parts of dicotyledonous plants (*Dicotyledones*) such as the seeds of *Hippocastani*, roots and flowers of *Primulae*, leaves of *Hedrae*, roots of *Ginseng*, bark of *Quillaja*, roots of *Glycyrrhizae*, roots of *Senegae*, leaves of *Polygalae Amarae*, roots of *Saponariae*, seeds of *Glycine max* and leaves of *Herniariae*. Legumes such as soybeans, beans and peas are rich sources of triterpenoid saponins. Steroidal saponins are typically found in members of the *Agavaceae*, *Alliaceae*, *Asparagaceae*, *Dioscoreaceae*, *Liliaceae*, *Amaryllidaceae*, *Bromeliaceae*, *Palmæ* and *Scrophulariaceae* families and accumulate in abundance in crop plants such as yams, alliums, asparagus, fenugreek, yucca and ginseng. Diosgenin, the steroidal aglycone obtained by hydrolysis of dioscin, a saponin abundant in the tubers of *Dioscorea villosa* (wild yam), is the precursor for commercial synthesis of steroids such as cortisone, progesterone and pregnenolone. Steroidal glycoalkaloids are commonly found in members of the *Solanaceae* family including tomato, potato, aubergines and capsicum [8]. Cereals and grasses are generally deficient in saponins, with some notable exceptions, such as the *Avena* species (oats) which accumulates both triterpenoid and steroidal saponins. The phylogenetic tree with plant subclasses from which saponins have been isolated and characterized is presented in **Figure 3**.



**Figure 3.** The phylogenetic tree with plant subclasses [16].

Some studies have suggested that variations in saponin distribution and composition in plants may be a reflection of varying needs for plant protection. In some plants, for example, *Phytolacca dodecandra* (gopo berry) and *Dioscorea pseudojaponica* (yam), maximal saponin accumulation has been noted during fruit and tuber development and has been suggested to protect reproductive organs. However, it was documented that in several plant species, the production of saponins is induced in response to biotic (herbivory and pathogen attack) and abiotic (humidity, nutrient starvation, light, temperature) stresses [8].

The main sources of saponins in human diet are legumes, mainly broad beans, kidney beans and lentils. Saponins are also present in *Allium* species (onion, garlic), asparagus, oats, spinach, sugarbeet, tea and yam. Nevertheless, the main plant sources of saponins used in medicine and industrial applications are soap bark tree (*Quillaja saponaria*), Mojave yucca (*Yucca schidigera*), licorice (*Glycyrrhiza* species), ginseng (*Panax* species), fenugreek (*Trigonellafoenum-graceum*), alfalfa (*Medicago sativa*), horse chestnut (*Aesculus hippocastanum*), soapwort (*Saponaria officinaux*), gypsophila genus (*Gypsophila paniculata*) and sarsaparilla (*Smilax* species).

Some of the better-known botanicals rich in saponins are presented in **Table 1**.

Plant		Saponin content [%]
Latin name	Common name	
<i>Aesculus hippocastanum</i>	Horse-chestnut	3
<i>Avena sativa</i>	Oat	0.1–0.13
<i>Beta vulgaris</i>	Sugar beet (leaves)	5.8
<i>Chenopodium quinoa</i>	Quinoa	0.14–2.3
<i>Cicer arietinum</i>	Chickpea	0.23
<i>Crocus savitus</i>	Saffron crocus	1.2–3.4
<i>Glycine max</i>	Soybean	0.22–0.49
<i>Glycyrrhiza glabra</i>	Licorice (root)	22.2–32.3
<i>Hedera helix</i>	Ivy	5
<i>Medicago sativa</i>	Alfalfa	0.14–1.71
<i>Panax ginseng</i>	Chinese ginseng	2–3
<i>Panax quinquefolius</i>	American ginseng	1.42–5.58
<i>Pisum sativum</i>	Green pea	0.18–4.2
<i>Polygala spp.</i>	Milkwort	8–10
<i>Primula spp.</i>	Primula	5–10
<i>Quillaja saponaria</i>	Quillaja bark	9–10
<i>Saponaria officinalis</i>	Soapwort	2–5
<i>Smilax officinalis</i>	Sarsaparilla	1.8–2.4
<i>Trigonellafoenum-graecum</i>	Fenugreek	4–6
<i>Yucca schidigera</i>	Yucca	10

**Table 1.** The better-known plants—sources of saponins [21, 22].

In Northern Europe, the main sources of saponins are: *Saponaria officinalis*, *Calendula officinalis*, *Salvia*, *Digitalis*, *Verbascum*, *Solanum* species, sugar beet, oats, etc.

*Calendula officinalis* (Asteraceae) is well-known medicinal plant in Poland. It is also popular in gardens as a decorative annual species. Traditionally, it has been used topically for many eruptive skin diseases and abrasions, as well as for gastric and menstrual discomfort, as a plant with antiseptic, mild diaphoretic and antispasmodic properties. Calendula contains significant amounts of oleananesaponins, which form two distinct series of related compounds, called 'glucosides' and 'glucuronides' according to the structure of the respective precursor. Extracts from marigold flowers are still used in ointments, cosmetic creams and hair-shampoos [15].

In sugar beet leaves, saponins have been reported at level of 5%, and in roots 0.1–0.3%. However, during raw beet processing, these saponins are mostly concentrated in the waste products. For example, the concentration of saponins in sugar beet pulp water reaches 1.2% [23]. Similar concentrations of saponins have been detected in the filtration residues and molasses. In Polish research laboratories, several triterpene-based saponin structures have been isolated and characterized [24]. Given the scale of worldwide sugar production from sugar beet, this plant can be considered as an industrial source of saponins [25]. Sugar beet as a high economic value crop will have a prosperous perspective of application in the food, bioenergy, and pharmacy industries [26].

In Southern Europe, the region around the Mediterranean Sea is rich in grapes. Saponin glycosides in red wine are known as heart protective, due to their LDL cholesterol-lowering and HDL cholesterol-increasing effects. The saponins in red wine also help prevent clumping of red blood cells. Many of plant species rich in saponins are used traditionally in Greece for making herbal teas, as flavorings and seasonings and have been tested for various pharmacological activities [27]. Mediterranean thyme (*Thymus capitatus*) is a common plant in the Mediterranean region, growing in arid rocky places and flowering between May and August. It is commonly used as a medicinal and culinary herb, owing to its strong and agreeable odor, mainly attributed to its essential oil. Other constituents include saponins and organic acids. Thyme has several medicinal uses including antiseptic, expectorant, antispasmodic and anthelmintic properties. Greek agave plants contain saponins and fructans. Many other representative species of the Mediterranean flora including *Melissa officinalis* (balm), *Origanum vulgare* (wild marjoram), *Origanum dictamnus* (dittany of Crete or hop marjoram), *Hyssopus officinalis* (hyssop), *Dioscorea villosa* (wild yam), *Viola tricolor* (wild violet, wild pansy, heartsease, Johnny jump-ups), *Salvia officinalis* (sage), *S. officinalis* (common soapwort), *Tribulus terrestris* (tribulus) contain saponins with antioxidant and anti-inflammatory properties and can boost the human immune system [28, 29]. The genus *Ruscus* (Asparagaceae family) is native to the Mediterranean, Southern and Western Europe. The underground parts of *Ruscus* plants are a source of steroidal saponins. *Ruscus* extracts were extensively used, especially in Germany and France, for the treatment of chronic venous insufficiency, varicose veins, hemorrhoids, and orthostatic hypotension [30].

China is rich in various plant sources of saponins, which are often unknown in Europe. *Mussaenda pubescens* (Rubiaceae), *Bupleurum chinense*, *Clinopodium chinense* var. *parviflorum* and

*Clematis chinensis* Osbeck (*Ranunculaceae*) and *Yucca elephantipes* are Chinese folk medicine plants used as diuretics, antiphlogistics, diaphoretics and antipyretic agents and have also been used to detoxify mushroom poisons and terminate early pregnancy. *Yucca* (*Agavaceae*) plants are native to China. The leaf extract of *Y. elephantipes* with saponins has been reported to have antiviral activity against tobacco mosaic virus and to exhibit antifungal activity against the pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans* [31].

The interesting plant in China is *Caragana*, also known as peashrub, a member of *Fabaceae*. More than 80 *Caragana* species were recorded, and several of them have a long history of use in traditional Chinese medicine, for example, in the treatment of cervical and breast cancer. Seeds of this legume represented an interesting source of triterpenoid saponins of the soyasaponin B type [32].

The *Glycyrrhiza* genus (*Leguminosae* family) consists of about 30 species and is widely distributed all over the world. In China, three species *G. uralensis*, *G. glabra* and *G. inflata* are officially used as licorice and recorded in Chinese Pharmacopoeia. Biological studies showed that licorice has a variety of biological effects, such as antioxidant, antiviral, anti-cancer, antidepressant, anti-inflammatory, anti-carcinogenesis, hepatoprotective and neuroprotective bioactivities [33, 34].

The important source of natural medicines is *Panax* genus. Three valuable *Panax* species *P. ginseng*, *P. quinquefolius*, and *P. notoginseng* are of great interest to medicine and food industry, and they are widely used in healthcare products, foods and food additives. To the end of 2012, at least 289 saponins were reported from eleven different *Panax* species [35]. Most of them are glycosides of triterpenoid aglycones [36]. Ginseng has been used as a herbal medicine in China for thousands of years due to its wide pharmacological properties, such as anticancer, antidiabetic, antifatigue, anti-ageing, hepatoprotective and neuroprotective [37]. It was also documented that *P. notoginseng* saponins suppress radiation-induced osteoporosis by regulating bone formation and resorption [38].

*Calamus leptospadix* grows as a non-climbing palm in the Sub-Himalayan region. Extract of *C. leptospadix* was characterized by Borah and co-workers, and they documented presence of a triterpenoid saponin with antimicrobial properties against both *Escherichia coli* and *Candida albicans* [39].

*Stauntonia brachyanthera* is an evergreen shrub belonging to the family of *Lardizabalaceae*, mainly distributed in the southwest of China. This plant is traditionally used to treat various diseases. Its fruit, zhuyaozi, is very popular in the southwest of China because of its fresh taste and abundant nutrients. The chemical study on this fruit resulted in the isolation of triterpenoid saponins. This research provided useful clues for the fruit of *S. brachyanthera* as a new resource of food for hepatoprotection [40].

*Camellia oleifera*, originated in China, is an important source of edible oil obtained from its seeds. This plant has been used as a natural detergent, and its extract rich in saponins is commercially utilized as a foam-stabilizing and emulsifying agent. The percentage of crude saponins extract that was obtained from the defatted seed meal of *C. oleifera* was 8.34% [41].



Plant saponins show region-specific character. It was found that variety of soybean from China is richer in saponins than those from Japan, Canada or United States [42]. *Tribulus terrestris* samples collected in Bulgaria, Greece, Serbia, Macedonia, Turkey, Georgia, Iran, Vietnam and India were analyzed by LC-ESI/MS/MS, and the results revealed distinct differences in the saponin profiles depending on region of sample collection, plant part studied and stage of plant development. The samples from Bulgaria, Turkey, Greece, Serbia, Macedonia, Georgia and Iran exhibited similar features but the Vietnamese and Indian samples exhibit totally different chemical profile. The obtained results suggested the existence of one chemotype common to the East South European and West Asian regions [43]. Studies conducted by Montero and co-workers showed that several licorice (*Glycyrrhiza glabra*) samples collected at different locations were characterized by specific metabolite profiles. Therefore, it was concluded that obtained 2D-chromatograms from the different licorice samples can be used as typical patterns that could potentially be related to geographical location and authentication of plant source [44].

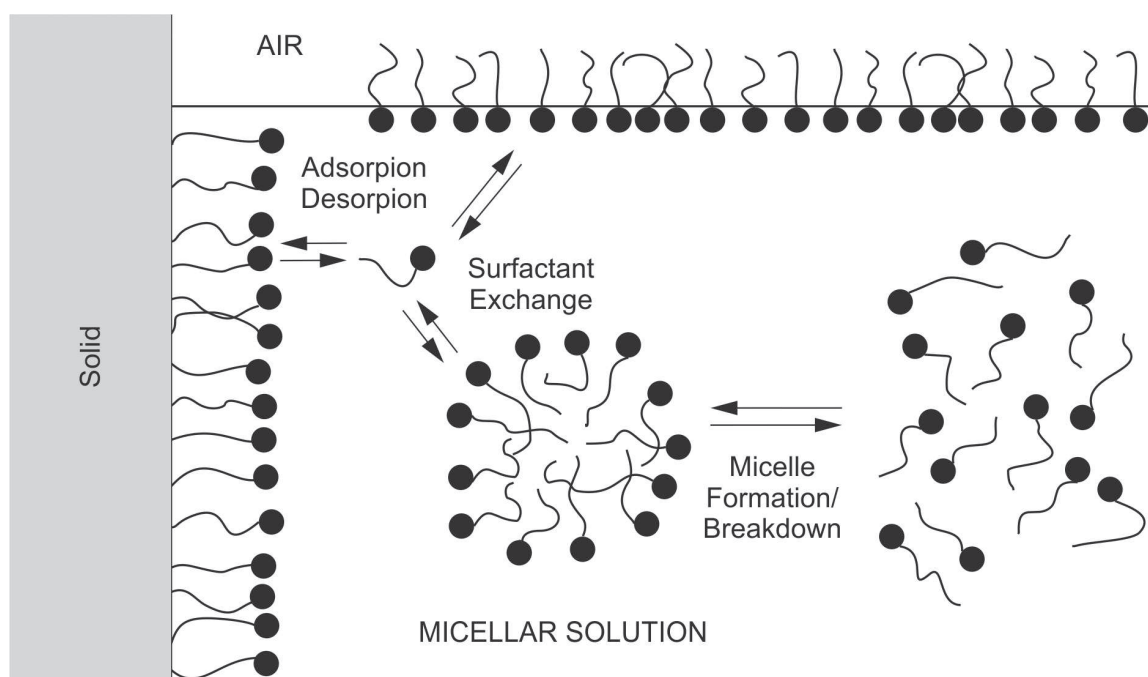
To obtain saponins from plant material different extraction methods may be used, using solvents as water, methanol, ethanol or hydroalcoholic mixtures in Soxhlet extractors or in orbital shakers. In addition, other solvents such as glycerol and aqueous or alcoholic surfactants solutions were also reported. Novel procedures use lower amounts of solvent but additional physical/chemical treatment: multi-stage extraction, pressure, microwaves, ultrasounds or supercritical fluid extraction. These methods can lead to an increase in the process efficiency. However, it should be considered that under harsher conditions (higher temperature and pressure), saponins can be hydrolyzed and degraded, so rather mild processes should be used [45–49].

#### 4. Natural surfactants and emulsifiers

Saponins, due to the presence of a lipid-soluble aglycone and water-soluble sugar chain, show amphiphilic nature. In this way, foam formation (with liquid-gaseous phases), an emulsifier effect (with liquid-liquid phases) and dispersion abilities (with liquid-solid phases) are achieved. Saponins with one sugar chain have the best foaming characteristics. The compounds with two or three sugar chains show decreasing of foaming ability. Some saponins without foaming character have also been observed [17].

In aqueous solution, saponin molecules align themselves vertically on the surface with their hydrophobic ends oriented away from the water. This has the effect of reducing the surface tension of the water, causing it to foam. In aqueous solutions, surfactants form micelles above a critical concentration called critical micelle concentration (CMC). Below this concentration, molecules remain unassociated. Micelles have a lipophilic center, and this creation of a fat-loving compartment explains why detergents can dissolve grease and oils (**Figure 4**).

The size and structure of micelles are dependent on the type of saponin. For example, saponins from *S. officinalis* and soya bean form small micelles consisting of only two molecules, while the aggregates of *Quillaya saponaria* saponin consist of 50 molecules. It was documented that the properties and the aggregation number (number of monomers) of micelles forming



**Figure 4.** Micelle formation [50].

by *Quillaya* saponins are affected by temperature, salt concentration, and pH level. For saponins from *Q. saponaria*, CMC is equal from 0.5 to 0.8 g/l at temperature 25°C and decreases with increasing salt dose [17]. The micelle shapes depend on the saponin molecule. For example, micelles formed by *Saponaria* and *Quillaya* saponins are elongated or even filamentous, while those formed by saponins of *G. max* are rather circular. Probably, the reason for these differences is the chemical structure of aglycone.

The presence of carboxylic acid in the saponin molecule may strongly influence the surface activity. Additionally, the location of this acid in the molecule is particularly important. For example, *G. max* saponin contains -COOH group in its hydrophilic part. The carboxylic group dissociates in aqua phase and forms free carboxyl anion, responsible for increasing the solubility of saponin in water environment. In contrast, saponins of *Sapindus mukorossi* (Chinese washnut) also contain the carboxylic groups but they attach to the hydrophobic aglycone. In consequence of this mechanism, the dissociation level of -COOH groups is very low. Saponins can also form mixed 'sandwich-like' or 'pile of coins-like' micelles with bile acids. These are much larger than the micelles of saponins alone, and they differ depending on the structure of the aglycone. In the presence of bile acids, saponins from *S. officinalis* and *Q. saponaria* form filamentous structures, while *G. max* saponins have an open structure. The ability of saponins to form large stable micelles with bile acids gives important implications for dietary mechanisms. Saponins in food and feed increase fecal excretion of bile acids. Additionally, the incorporation of cholesterol into saponin micelles increases their size, CMC, viscosity, and the aggregation level resulting in the solubility enhancement of cholesterol. The micelles formed are too large for the digestive tract to absorb. This mechanism leads to decreasing of the plasma cholesterol concentration. Saponin *Q. saponaria* was found to solubilize cholesterol significantly better than linear hydrocarbon chain surfactants [51].

Interactions between saponin and membrane-bound cholesterol lead pore formation and increasing of membrane permeabilizing properties. This specific effect of saponins depends on the combination of various factors: the membrane composition, the type of saponin, and—especially—the nature of aglycone [52].

Saponins also affect the permeability of intestinal cells by forming complexes with sterols in mucosal cell membranes. This leads to increase in intestinal mucosal cells permeability. Thus, this facilitates the uptake of substances to which the gut would normally be impermeable, for example, milk allergen  $\alpha$ -lactoglobulin [17].

*Quillaja saponins* also had a solubilizing effect on some toxic polycyclic aromatic hydrocarbons, which increases linearly with saponin concentration at values higher than CMC. A similar linear correlation has been observed between the concentration of the saponins from *Sapindus mukorossi* and aqueous solubility of hexachlorobenzene and naphthalene [21]. Saponins also enhance solubility of Yellow OB, and progesterone [8] Purified saponins and saponin mixtures resulted in both enhancements and reductions in water solubility of quercetin, digitoxin, rutin and aesculin [53].

Emulsifiers play two key roles in the creation of successful emulsion-based products. They: (i) facilitate the initial formation of fine lipid droplets during homogenization and (ii) enhance the stability of the lipid droplets once they have been formed. Oil-in-water emulsions may be formed using either high- or low-energy approaches. High-energy approaches utilize mechanical devices (homogenizers): high shear mixers, colloid mills, high-pressure valve homogenizers, microfluidizers, and sonicators. Low-energy homogenization relies on the spontaneous formation of emulsions when the composition or environment of certain emulsifier-oil-water mixtures is changed in a particular way. *Quillaja* saponin is a natural effective emulsifier to form and stabilize oil/water emulsions with very small oil beads ( $d < 200$  nm). They are stable in wide range of environmental parameters (pH, ionic strength, temperature). This fact makes saponins of *Q. saponaria* suitable for wide application in food products [54].

*Quillaja* saponins currently find commercial scale in food industry as emulsifiers with milk and egg proteins, for example,  $\beta$ -lactoglobulin,  $\beta$ -casein or egg lysozyme by electrostatic and hydrophobic interactions as well as by specific sugar binding sites [55].

## 5. Biological activity

Due to their amphiphilic nature, saponins show a wide range of biological activities. Various crude isolates, extracts, and saponin containing plants were utilized in the investigation of biological activity in the earlier studies; however, progress in the isolation/purification and characterization techniques has enabled the investigation of the bioactivity of well characterized [56, 57]. Saponins have been shown to swell and rupture erythrocytes causing a release of hemoglobin. The effect of saponin on erythrocyte death or hemolysis may limit the therapeutic use of the substances. On the other hand, saponins have been proposed for the treatment of a variety of diseases, including diabetes, obesity and osteoporosis [58]. Pharmacological

effects of saponins include stimulation of immune responses. Their efficacy against cancer has been attributed to their ability to inhibit cell proliferation, to counteract angiogenesis and to stimulate apoptosis [59–61].

The toxicity of saponins to insects (insecticidal activity), parasite worms (anthelmintic activity), molluscs (molluscicidal), and fish (piscidal activity), and their antifungal, antiviral, and antibacterial activity is well documented. Toxicity of saponins to warm blooded animals is dependent on the source, composition, and concentration of these compounds. The results of in vivo studies with rats, mice, and rabbits implied that saponins are not absorbed in the alimentary channel but hydrolyzed enzymatically to sapogenins [21].

The action of saponins, by enhancing the immune response to antigens, has been documented since 1940s. *Quillaja* saponins are exclusively used in the production of saponin adjuvants, and this immune function was also reported for soya, quinoa, gypsophila and *Saponaria* saponins [62]. Due to the structural complexity and toxicity of plant saponins, their use in human vaccines is limited, but the progress in new processing and purification techniques with maintaining of immunological adjuvant activity is important to create saponins as new generation vaccines [63].

Several mechanisms have been proposed to explain the hypocholesterolaemic activity of saponins. Possible mechanisms may involve the capacity of saponins to: (i) form insoluble complexes with cholesterol, (ii) affect micelle formation, (iii) interfere with bile acid metabolism, (iv) inhibit lipase activity, or (v) regulate cholesterol homeostasis via monitoring the expression of the key regulatory genes of proteins or enzymes related to cholesterol metabolism [58, 64]. Cholesterol-lowering activity of saponins has been demonstrated in both animal and human trials. Animal diet containing purified saponins or concentrated saponin extracts containing, for example, digitonin (saponin from *Digitalis purpurea*), saikosaponin (saponins from *Bupleurumfalcatum* and related plants) and saponins from *Saponaria*, soya, chick pea, *Yucca*, alfalfa, fenugreek, *Quillaja*, *Gypsophila*, and garlic resulted in reductions of cholesterol concentrations [21].

Anticancer activity has been reported for soya saponins, ginsenosides, saikosaponin, diosgenin and glycyrrhizic acid. In particular, the potential of soybean saponins as anticarcinogens has been studied in recent years. Anticancer activities of saponin containing plants such as ginseng and licorice were also investigated [65].

The study of the relationship between chemical structure of aglycones and colon anticancer activity of soybean saponins revealed that the soya sapogenols were more bioactive than the glycosidic saponins. Other aglycones with anticancer activity include dammaranesapogenins from ginseng, betulinic acid, and oleanolic acid. These two last compounds were also reported to possess anti-viral, anti-inflammatory, hepatoprotective, anti-ulcer, antibacterial, hypoglycaemic, anti-fertility, and anticariogenic activities. However, the conversion of saponins to their aglycones may also result in the loss of activity. For example, the hydrolysis of saponins by ruminal bacteria results in the loss of antiprotozoal activity. Similarly, the deacylation of *Quillaja* saponins decreases their adjuvant activity [66].

## 6. Antimicrobial activity

The antimicrobial effects of saponins extracted from plants have been studied in *Solanum*, oats, seeds of *Capsicum annuum*, alfalfa, garlic, *Yucca*, *Quillaja*, etc. The saponin extracts were tested against numerous Gram-positive and Gram-negative bacteria, yeasts and molds. However, the results were varied due to the high diversity of plant saponins [67]. For example, saponins from *Yucca* exhibit antimicrobial activity against Gram-positive cells but do not act on Gram negative bacteria. However, *S. officinalis* extracts showed antibacterial action against Gram negative, avian pathogenic *Escherichia coli* (APEC) strains [68, 69]. In general, the antibacterial activity of saponins is often weak, whereas significant antifungal activity has been observed. The primary mode of action of saponins toward fungi involves pore formation and loss of membrane integrity. The mechanism of action is an analogous to hemolytic activity of saponins. It was proposed a model of action for avenacin—triterpene saponin of oats. The first step involves the insertion of the aglycone fragments into the membrane and then their binding to sterols [70]. The following stage conducts to the interaction of sugar residues and formation of sterol-saponin complexes. This phenomenon leads to the rearrangement of membrane lipids, formation of pores and—finally—lysis of cells [71, 72]. Yeast studies on *Quillaja* saponins conducted in Poland found that saponin treatment lead to increased cell membrane permeability in different yeast strains, and therefore, it was concluded that *Quillaja* saponins facilitate the process of obtaining yeast salt-free lysates [73]. It is interesting that *Yucca* and *Quillaja* saponins increased growth of bacterial *Escherichia coli* cells up to a certain concentration, and thereafter decreased growth [74]. Arabski and co-workers demonstrated that saponin *Q. saponaria* at dose of 12  $\mu\text{g}/\text{mL}$  enhanced the six *E. coli* strains growth [75]. Naturally, cholesterol-free Gram-negative bacteria cell-wall outer membranes are around 90% covered with lipopolysaccharide (LPS). Therefore, it was concluded that saponin may interact with the lipid A part of LPS and thereby increase the permeability of the bacterial cell wall. Sublethally injured or weakened cells may become more susceptible to the action of conventional disinfectants, even at reduced concentrations. It was suggested that lipid A-saponin complexes could promote antibiotic (colistin, ampicillin) or disinfectant action toward inherently resistant microbial cells [75]. The similar results were obtained by Alberice and co-workers [76]. They documented that application of saponin extract in the food industry would be economically viable and sustainable. The results indicated that saponin alone can be used by the industry as a bactericide to reduce the risk of juice spoilage by Gram-positive cells *Alicyclobacillus acidoterrestris*.

## 7. Commercial applications

*Y. schidigera* and *Q. saponaria* are the two major commercial sources of saponins added to cosmetics as well as food products as emulsifiers and long-lasting foaming agents [17].

*Y. schidigera* is a native plant from southwestern United States and Mexico. Native Americans used it to make soap. The trunk of the plant is mechanically shredded, and yucca juice is produced by mechanical squeezing in a press. The obtained juice is concentrated by evaporation. *Y. schidigera*

syrup (concentrated juice; *Yucca* extract), and dried and finely powdered logs (*Yucca* powder) are of particular interest to cosmetic, pharmaceutical and beverage industries as well as animal nutrition [77]. These products possess foaming features that are of particular interest in cosmetic, soft drinks (root beer), food and feed industries [78].

In the United States, *Yucca* is listed in The Code of Federal Regulation [79]. In Japan, *Yucca* extract (extract of whole plant of *Yucca arborescens* or *Y. schidigera*) is listed in the List of Existing Food Additives [80]. Because steroidal saponins in *Yucca* exhibit antifungal activities, *Yucca* extract has been added to food as a 'shelf life extender' in the Japanese market. *Yucca* powder water extracts can be successfully used in confectionery/food industries for improving both product quality and shelf stability. Sucharzewska and co-workers documented that *Yucca* extract contains two groups of beneficial substances. One group is formed by steroidal saponins, which may improve product quality (porosity, density, and hardness), and the second one is created by antioxidants that are able to reduce fat oxidation and extend food quality during shelf-life time [81]. It is also worth to note that *Yucca* extracts may be used as natural, non-toxic deodorizers. The studies conducted in Poland show that combined treatment with microbial preparations and *Yucca* extract can significantly reduce the concentration of odorants in poultry manure [82]. Natural saponin extracts, namely those that may be obtained by steam treating the pulp of *Yucca* with water, in combination with proteins exhibit a synergistic effect, eliminating odors from the breath and oral cavity of humans, as well as from other environments [83].

Tenon and co-workers used HPLC/ELSD technique for *Yucca* steroidal saponin quantification. This method is effective for routine industrial analyses for saponin fingerprints and capable of distinguishing saponin profiles from taxonomically distant species [78].

The second saponin source of commercial value is *Q. saponaria*. The term 'quillaia' refers to the dried inner bark of the tree, which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, principally Bolivia, Chile, and Peru [84, 85]. The bark of this tree was used as shampoo in for hundreds of years. *Quillaja* extracts contain over 100 triterpenoid saponins. The basic structure of them is the hydrophobic triterpenoid quillaic acid known as sapogenin, and the hydrophilic sugar moieties are attached at two positions: di- or trisaccharide at C3 and oligosaccharide at C28 [85]. Young plants usually exhibit less heterogeneous saponins profiles than those obtained from mature extracts [87].

A large amount of *Quillaja* saponin is mainly utilized as a surfactant. It is also used in beverages, food ingredients, shampoos, liquid detergents, toothpastes and extinguishers as an emulsifier and long-lasting foaming agent. Additionally, a saponin mixture possessing immune-adjutant properties was given a pharmaceutical application, as a suspension stabilizer [88].

The beneficial effects of extracts from *Yucca* and *Quillaja* are well documented. The extracts from these plants may influence microbial fermentation. Inhibition of gut microbes, particularly *Streptococcus bovis*, *Butyrivibrio fibrisolvens*, *Escherichia coli* and rumen protozoa has been reported [74]. Extracts of *Y. schidigera* and *Q. saponaria* have been used as 'food grade' saponins. This term is widely used by manufacturers, and it is defined as any grade or preparation of saponin which is approved for use in food and beverages under the United States Food and Drug Administration (FDA).

According to the Codex Alimentarius Commission, extracts from *Q. saponaria* may be used as a foaming agent in 'water-based flavored drinks', including 'sport' or 'electrolyte' drinks and particulate drinks (GSFA category 14.1.4, 500 mg/kg maximum use level). In soft drinks, unpurified *Quillaja* extracts are used at dose up to 200 mg/kg. However, in syrups intended for dispensable frozen beverages (FCBs) or frozen lemonades, *Quillaja* extracts may be up to 500 mg/kg on dry solid basis [87].

Although *Quillaja* and *Yucca* saponins are not considered Generally Recognized As Safe (GRAS) by FDA, they have been assigned as GRAS by Flavor and Extract Manufacturers' Association of the United States (FEMA) with FEMA number 2973 [21, 87].

**Quillaja** extracts are classified as type 1 and type 2 based on their saponin content, 20–26% and 75–90%. *Quillaja* extract, type 2, is used in Japan as an emulsifier for preparations containing lipophilic colors or flavors that are added to soft drinks, fermented vegetables, and dressing [87]. Other saponins used food additives include enzymatically modified soybean saponin, *Pfaffia* and *Yucca* extracts, and tea seed saponins [80].

In the European Union, *Quillaja* extract is classified as the foaming agent for use in water-based, flavored non-alcoholic drinks and labeled as E999 (200 mg/l calculated as anhydrous extract) [87].

The physiochemical properties of saponins can also be utilized in food processing applications, thus, while complex formation of saponins with cholesterol has been used for the removal of cholesterol from dairy products such as butter oil [89–91]. It was documented that the natural food-grade surfactant isolated from the bark of the *Q. saponaria* Q-Naturale® may be able to replace synthetic surfactants in food and beverages [92]. The interaction of saponins with cell membranes has been considered for the selective precipitation of fat globule membranes from cheese whey. In this application, saponins are used to increase the hydrophobicity of the fat membrane to facilitate flocculation and precipitation of the formed complexes.

As a natural surfactant, *Q. saponaria* saponins demonstrated good performance in manufacturing orange oil nanoemulsions. This fact may permit the manufacture of good quality orange oil-based nanoemulsions in beverage and alcohol-free mouthwash applications [93, 94]. *Quillaja* saponins show a high surface activity and functionality to solubilize a lutein ester extract for its incorporation in food matrices [86]. Additionally, it was documented that the mixtures containing *Quillaja* saponins and lecithins were rather unaffected upon heating from 25 to 75°C. Therefore, these results provide important insights into selecting surfactants to be used in specific food applications, for example, whether the food will be heat treated or not. This type of structure modulation through different environmental conditions and heating may also be useful for structure design in pharmaceutical applications [95].

Dried roots of licorice represent an important agricultural product. The name 'glycyrrhiza' originates from the Greek words 'glykosrhiza,' which mean 'sweet root.' Licorice is used as a sweetener and a flavor enhancer for foods in China and other countries. It is approved by Food and Drug Administration USA as a food additive, regarded with the 'GRAS' label and registered as CFR 184.1408 [33].

Saponins can be used to enhance both the effectiveness of cleaning/disinfection processes. They are considered natural detergents and are used as additives in washing powders, and additives for liquid/powder cleaning. The addition of a small amount of a saponin to an aqueous environment provides a product that is an effective water clarifier and solid surface cleanser. These compositions may be used to clean metals, metal-plated surfaces, ceramics, wood, glass, etc. The use of natural plant products as detergents could provide cheaper, safer and more consumer-acceptable alternatives to synthetic compounds.

## 8. Conclusion

Saponins are diverse compounds traditionally used as natural detergents. Their physico-chemical and biological properties are wide exploited in food, cosmetics and pharmaceuticals. Information on the composition (qualitative and quantitative), properties of the saponins present in the raw material, and the effects of processing on their composition and properties are key elements of successful process design.

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