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AN ANALYSIS ON SAPONINS FROM THERAPEUTIC PLANTS:
CHEMISTRY, SEPARATION AND DETERMINATION

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Abstract

Saponin isolated from medicinal plants is a naturally bioorganic patch with high molecular weight. Its aglycone (water-non-soluble part) nucleus has 27 to 30 carbon atoms besides one or two sugar moieties (water-soluble part) containing at least 6 or 12 carbon atoms independently. The complexity of saponin chemistry is perhaps a gap for numerous scientists and experimenters to understand the relationship between the chemical structure and its medical or pharmaceutical behaviour. Lately, the increase in demand for saponin operations was observed due to colourful natural, curative, and pharmaceutical bearing. Accordingly, this present reexamination article provides detailed information about the chemistry of saponin, especially triterpenoid saponin. Groups, chemical structure, the possible traditional isolation practices, qualitative and quantitative determination of saponins were included. Exemplifications of the mono and bidesmosidic structure of oleanolic acid and hederagenin were also outlined. Structural differences between triterpenoid, steroid, and alkaloid glycosides were abstracted corresponding to their atoms, rings, and functional groups.

Keywords: Saponin, chemistry, isolation, medicinal plants, extraction, determination, triterpenoid, a steroidal saponin, alkaloidal saponin

Introduction

It has been estimated that out of 4,22,000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes. Since ancient times, bioactive medicinal plants have been used in traditional or folk medicine to treat various diseases. Recently using phytochemicals has been considered safer and congenial to the biology of the human body. Medicinal plants are the primary source for preparing and extracting various modern drugs and pharmaceuticals like saponins. Signs of the progress of Phytochemistry have been supported enormously by the rapid development and accumulation of chemical methods of screening various medicinal plants for particular biochemical usage. The pharmaceutical and medicinal values of the applied medicinal plants are in the bioactive phytochemical constituents that produce specific physiological action on the human body. Some of the most important bioactive constituents are saponins, flavonoids, and alkaloids. Triterpenoid saponins are a prehensive, biologically active group of terpenoids and include a large chemical diversity of secondary metabolites with more than 100 carbon skeletons identified from terrestrial, marine living organisms, and medicinal plants. Triterpenoids as saponin have their characteristics. They cause hemolysis

of red blood cells (RBCs), form persistent froth if shaken with water, and are soluble in water, alcohol, and a mixture of both. These naturally occurring compounds form the backbone of modern medicine or drugs. Saponins are a class of bioorganic compounds found in particular abundance in the plant kingdom. More specifically, they are naturally occurring glycosides described by the soap-like foaming, and consequently, they produce foams when shaken in aqueous solutions.

Structurally saponins have one or more hydrophilic glycoside sugar moieties combined with a lipophilic triterpene molecule. Literature shows that saponins exhibit a biological role and medicinal properties. Saponins were considered a precursor for the semi-synthesis of steroidal drugs in the pharmaceutical industry. Sheng et al. reviewed the clinical significance of triterpenoid saponins in preventing and treating metabolic and vascular diseases. The above medicinal research and applications reflect the interest in saponins as bio-natural source material. However, understanding the relationship between saponins' chemistry and its medical action is not easy for many chemists, physicians, and researchers. Due to the complexity, importance, and interferences of the chemistry of saponin, the primary purpose of this review article is to give detailed information.

- (1) The classification of saponin,
- (2) The chemical structure of various types of saponins and their functional groups,
- (3) Examples for mono and bidesmosidic triterpenoid saponins,
- (4) Ordinary and Soxhlet extraction methods in the laboratory, and finally
- (5) Qualitative and quantitative determination of saponins.

Chemistry of saponins:

Saponins are naturally occurring bioorganic compounds having at least one glycosidic linkage (C-O-sugar bond) at C-3 between aglycone and a sugar chain. Hydrolysis of saponin molecule produces two portions, aglycone and a sugar moiety. Isolated amorphous solid saponins have a high molecular weight and contain 27 to 30 carbon atoms in the non-saccharide portion.

Partition (A): Non-saccharide

The non-saccharide portion (the hydrocarbon skeleton part without a sugar chain) is genin, sapogenin, or aglycone. Depending on the type of sapogenin present, the saponins can be divided into three major classes:

Triterpenoid glycosides:

This type of saponins is the most widely distributed in the plant kingdom. Triterpene means three monoterpenes (10 carbon atoms) of 30 carbon atoms distributed as six isoprene molecules. Triterpene and triterpenoid glycosides are pentacyclic compounds with 30 carbon atoms and no spiro-carbon atoms (one carbon common in two rings). Triterpenoid glycosides contain 4 oxygen atoms and usually have one hydroxyl group (OH) at C-3 and carboxyl group (-COOH) at C-28. One oxygen atom presents in the molecule as ether-linked oxygen at C-3, the two oxygen atoms present as ester-linked oxygen at C-28.

In contrast, the remaining oxygen atom presents as a nonattached alcoholic group (-CH₂-OH) at C-24. According to the number of sugar moieties attached to the aglycone nucleus, triterpenoid saponin can be classified into two types monodesmosidic and didesmosidic. Mono and didesmosidic structures of oleanolic acid and hederagenin as triterpenoid saponin were presented. Monodesmosidic triterpenoid glycosides have a single sugar chain, usually attached at C-3. Bidesmosidic triterpenoid glycosides have two sugar chains, often with one attached through an ether linkage at C-3 and the other either attached through an ester linkage at C-28 or through an ether linkage at C-24. Steroid glycosides are modified triterpenoids with the structure of tetracyclic six-membered rings and bicyclic five-membered rings containing 27 carbon atoms. Steroid glycosides have two hetero rings, one of both is a furan ring, and the other is a pyran ring. One spiro-carbon atom is ordinary between the two hetero rings (furanose and pyranose rings). Steroidal saponins are used mainly as precursors for the partial synthesis of sex hormones. Alkaloid glycosides it's the third class of non-saccharide portion of saponins. Alkaloid glycosides have a steroid-like structure, but alkaloid glycosides have a piperidine ring (six-membered ring containing N-atom) instead of a pyranose ring (six-membered ring containing O-atom) in steroid glycosides.

Partition (B): Saccharide moiety

It has a variety of pentoses (furanose ring) or hexoses (pyranose ring) sugars. It may be Dextro (D) or Leavo (L) isomers of and anomers (conformation at C-1 of the sugar). Simply the two saponin portions (aglycone and sugar moieties) can be designated found that oleanolic acid (Ole) and hederagenin (Hed) are two triterpenoid aglycones attached to a variety of sugar moieties forming mono, di, tri, and tetra-saccharide saponin structures. Rhamnose, arabinose, xylose, glucose, and ribose are the most hexoses sugar present in saponins. The three categories of saponins can be formed and symbolized.

Extraction and isolation of saponin

Due to the increase in public awareness of preventative health care, an intensive review on the recent advances in extraction of a bioactive compound from medicinal

plants have been reported in references. Almost all kinds of literature explain three different techniques for the extraction of saponins. Ordinary extraction and soxhlet extractor are well-known extraction methods, while modern techniques like ultrasound-assisted extraction, microwave-assisted extraction, and accelerated solvent extraction methods are still in advances and enhancements. According to Choon YC, maceration, reflux, and soxhlet extraction represent about ~60% of the employed techniques in extracting saponins from plant materials. Modern extraction processes represent about ~30 %, while subsequent extraction method represents about 10% of the employed techniques. Therefore, attention is paid to the traditional extraction techniques in our present review.

Ordinary extraction

The extraction of saponins by maceration is the famous method using ordinary solvent-like alcohols and n-butanol. It is a solid-liquid interface extraction where saponin's compounds inside the plant material can easily extract by immersion or soaking the plant materials in a suitable specific solvent for some time with or without stirring or shaking. The polarity of the solvent, temperature, maceration time, solubility of saponins and its effective diffusion in the liquid phase are the main operational variables affecting the efficiency of the normal extraction process. The polar saponins dissolve in polar solvents, and the nonpolar compounds dissolve in nonpolar solvents. The diffusion rate of saponins into the liquid phase depends upon the rate of mass transfer of a solute from the plant material to the solvent. The concentration gradient between the solid and liquid phases is the driving force of the diffusion of saponins into the solvent. The common maceration technique is straightforward and does not need a sophisticated experimental setup. Ethanol C₂H₅OH, methanol CH₃OH, acetone CH₃COCH₃, ethyl acetate CH₃COOC₂H₅, dichloromethane CH₂Cl₂, and a mixture of solvents are the ordinary solvents used for the extraction of saponins from plant material, but ethanol (50-98 %) and n-butanol CH₃(CH₂)₂CH₂OH are the commonly used solvent. Occasionally, the maceration time varied from a few hours to a few days (3d), 6weeks are the longest recorded time, and 30 minutes are the shortest recorded time of ordinary extraction. Maceration of plant materials by organic solvents may be accelerated or facilitated by heat, shaking and magnetic stirring. The temperature of extraction varies from ambient to the boiling point of the chosen solvent, and the number of extracted materials also varies from a few grams to a few kilograms.

The amounts of isolated saponins function of time of extraction, temperature, shaking, and the amount of original material. The longer extraction time, the higher temperature, and the heavier the extracted mass with shaking, the more isolated saponin will have and vice

versa. The amount of used solvent or extractant did not specify adequately and varied from a few millilitres to a few tens of litres. Standard column chromatography with silica gel stationary phase and an organic solvent as a mobile phase consumes more solvents than the static maceration process. Various saponins with different chemical structures can be extracted by maceration from the wild, desert, and cultivated plants with various species. After maceration, the alcoholic crude extract of plant materials then evaporated to obtain a more concentrated saponin containing solution. This solution may dilute with water and be subjected to a solvent extraction process using n-butanol and a separating funnel. Finally, n-butanol is easily removed using a rotatory evaporator under vacuum, and the remaining saponin residue stays in the round-bottomed flask. The dry residual saponin material can be fractionated and identified using several techniques, e.g., column chromatography, Sephadex, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). The most commonly employed solvent system for TLC is chloroform glacial acetic acid – methanol-water (60:32:12:8) and ethyl acetate formic acid glacial acetic acid-water (100:11:11:26). Methanol water system (MeOH H₂O) is the standard solvent system used with HPLC.²⁸ The extraction and identification of saponin from the plant material are not easy and described as tedious.

Soxhlet extraction

The distillation process of plant material by Soxhlet is faster than the normal maceration process because it involves heating the organic solvent to its boiling point and then returning the condensed vapours to the original flask after passing through the plant tissue in the condenser. Hence, the extraction process takes place via the direct contact between the plant tissue and the hot fumes of the solvent. After a considerable extraction time, the colourless solvent becomes a dark green solution due to the mass transfer into the solvent. Then the solution was dried by rotary evaporator to dryness to obtain the dry crude extract of the plant, suspended in water, extracted by n-butanol and fractionated as mentioned above. Still, the solvent's polarity, extraction time, and temperature are significantly affected by soxhlet extraction. The normal maceration process is static extraction, but Soxhlet is dynamic extraction due to solvent circulation during extraction. The powder of the plant may initially be defatted with 60-80°C petroleum ether or n-hexane statically or dynamically until the fatty components have been removed before the final extraction.

Qualitative determination of saponins

Saponin tests were carried out on the plant materials using standard procedures as described in references. The common methods of screening for the presence of saponins in the plant material depend on the three

significant saponin characteristics (1) the chemical characteristics and the formation of stable foam with shaking, (2) the hemolytic action of saponin on blood, and (3) the surface-active properties of saponin. The occurrence of saponins can be determined and confirmed qualitatively in the laboratory by different saponin tests. Standard foam test: 3g of each dry plant powder were weighed and extracted with 300ml of hot distilled water in a beaker. After filtration, the aqueous extracts were cooled, stirred and stored at 4°C in an automated refrigerator for 24h.

About 5ml of the plant extract was transferred into a test tube and diluted with 5ml of distilled water. The mixture was shaken vigorously for 2 minutes. The persistent appearance of foam lasting for at least 15 minutes and the forming of an emulsion when olive oil was added confirmed the presence of saponins. Wet foam test: The test solution was diluted by water and shaken vigorously for 12min is stable foamy lather appeared in the top of the test tube of the sample. Dry foam test: About ≈5 grams of crude powder of the plant was shaken with 5 ml distilled water in a test tube and warmed in a water bath is stable, persistent froth, was mixed with 3 drops of olive oil and shaken vigorously. The formation of emulsion indicates the presence of saponins. Foam test for fresh samples: About ≈ 2gram of fresh plant sample (leaves) was added to 20ml distilled water (w/w = 1:10), mixed by electric mixer, the mixture was filtered, the filtrate was concentrated by evaporation in a water bath to half of the original volume, then transferred into a test tube. The stable, persistent froth was mixed with 3 drops of olive oil and shaken vigorously than observed for the emulsion formation, indicating the presence of saponins.

Quantitative determination of saponins

In this method, saponins are traditionally extracted into water/ethanol mixtures, after which the alcohol is removed by evaporation and the saponins extracted from the water phase into n-butanol. According to the reference, saponin can be determined quantitatively by taking the dry powder of the plant sample weighing about 5g and poured into 200ml of 20% ethanol solution. The suspension was heated over a hot water bath for 3-4h with continuous stirring at about 55-60°C. The mixture was filtered, and the plant powder's solid residue was re-extracted with another 200ml of 20% ethanol solution. The two combined solutions were evaporated over a water bath at about 80-90°C to reduce the volume to 40ml. The concentrated solution was transferred into a 250ml separating funnel, and 20ml of diethyl ether was added and shaken vigorously to remove impurities from the original solution. The aqueous layer was recovered for another extraction, while the ether layer with impurities was discarded.

$$\text{Total saponin contents, (\%)} = \frac{\text{weight of the saponin residue}}{\text{weight of the original plant material}} \times 100$$

The purification process was repeated after that 60ml of n-butanol was subsequently poured twice, and the combined n-butanol solutions (120ml) were washed twice with 20ml of 5% aqueous sodium chloride. The remaining aqueous solution was transferred to a dried pre-weighed porcelain crucible and dried in a drying oven at 60°C to a constant weight. The remaining residue is the saponin product, which can be calculated by the A large quantity of plant material is used to increase the isolated saponin contents. The process seems efficient but has many technical and environmental disadvantages, like using three organic solvents (alcohol, ether, and n-butanol) with remarkable amounts, the need for heating, drying, and time-consuming. Another method for the determination of total saponin content was reported, in which the dry aerial parts of the plant (5.0g) were defatted twice with petroleum ether (60-80°C) (2x50ml), an alcoholic solution (75%, 150 ml) was added to the defatted phase. Then, the mixture was refluxed at 70°C for four hours, and the extract solution was filtered and evaporated at 40-50°C in rotavapor. The dry residue was dissolved by a suitable amount of distilled water and extracted triplicate with n-butanol (3x40ml). The combined n-butanol solution was evaporated at 90°C using rotavapor to dryness, and the yield of saponin was calculated according to the equation.

Conclusion

This assessment shows that saponin has two main parts: the aglycone and a sugar moiety. Saponin with one sugar moiety was known as monodesmosidic, but with two sugar moieties was called bidesmosidic. According to the number of carbon atoms, the number of oxygen and nitrogen present in the molecule, aglycone or genin, or sapogenin itself was categorized into triterpenoid, steroid, and alkaloid glycosides. Maceration in organic solvent and Soxhlet is the primary traditional isolation way of saponins. The presence of saponin in the plant material may be confirmed using a dry or wet test depending on the foam formation characteristics. Determination of total saponin contents can be proceeding via consecutive solvent extraction by n-butanol.

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