Rev iew Article

# Extraction, Estimation and Thin Layer Chromatography of Saponins: A Review

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**ABSTRACT**

Since time immemorial saponins are in use in various parts of the world for sure shot treatment or for approximate and rudimentary treatment of various ailments. Till date they are among the most fancied natural chemical for scientist of various backgrounds (botanist, biochemist, pharmacognosist, pharmacologist, natural product chemist etc), because they are one of the most potent and promising bioactive chemicals, obtained from natural sources. Various saponins have been reported in literature for range of pharmacological activities. Worldwide drug discovery is going through a rough patch. Various models are being discussed, new approaches are emerging, and synthetic drugs are enjoying good position among available options, still natural sources can never be undermined, as most of the established drug molecules trace their route to natural sources directly or indirectly. Many naturally available saponins are being used in treatment of diseases. In this review article an attempt has been made to compile basic and crucial information (extraction methods, thin layer chromatography (TLC) and estimation) on putative secondary metabolite, saponins. Compiled information is highly concise and widely reported.

**Keywords:** Saponins, extraction, estimation, TLC, drug discovery, natural products.

## INTRODUCTION

Plant remains to be the enviable source of molecules of therapeutic significance. Since antiquity, these bio resources have been in use for variety of diseases in different part of the world. Regardless of the type of plant, targeted ailment or other such parameters, the one step which is one of the most important and common is, removal of the molecule or fraction or part thereof from the plant biomass. There are several extraction procedures or schemes (depending on various factors) for isolation of various plant constituents generally known as primary and secondary metabolites, nonetheless there are only one or two methods for scrupulous and perfect extraction of these metabolites. Irrespective of the plant or part thereof or activity or subsequent operation, these methods are sufficient to provide perfect extraction of various metabolites viz alkaloids, flavonoids, tannins, saponins, carbohydrates etc. Several new methods besides the usual organic solvent extraction have been developed over the last few years for the extraction of primary and secondary metabolites. These are alcohol extraction with various biocompatible solvents, recovery of carboxylic acids and antibiotics with reactive extraction, dissociation extraction, aqueous two-phase extraction, and supercritical and near critical fluid extraction. Extraction and re-extraction processes are integrated into a single step by emulsion liquid membrane and solid supported liquid membrane extractions[1]. In various publications, sometimes extraction schemes is not fully mentioned or not followed as mentioned in the pioneering text source or there is reporting of some modified process. There is a need of piled up information for the extraction, estimation and chromatography of some class of phytoconstituents, especially for the researchers interested in exploring a plant afresh or even for a routine assignment[1, 2]. Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin). Sapogenin portion is found to link with one or more oligosaccharide moieties by glycosidic linkage. Glycone portion consists of pentoses, hexoses, or uronic acids. The presence of both, polar (sugar) and non-polar (steroid or triterpene) groups bestow saponins with strong surfactant like properties. This surfactant behavior lends saponins properties they are known for, e. g. hemolytic, medicinal or other intrinsic properties[3]. This concise paper is an attempt to amass and summarize the most relevant and time tested procedures for three basic operations (extraction, TLC and quantitative estimation) while studying a plant from view point of phytochemistry or some allied reasons when it comes to saponins. To keep the text relevant and limited, barring few instances direct methods are given. Although many more procedures can be spotted in

literature, but extensively cited procedures are being mentioned here. Variation might be in starting solvent or fractionation schemes but in most of such cases ultimate steps usually remains same. Sometimes extraction is done to get rid of unwanted material for they hinder the removal of other metabolite or they are to be separated later in the extraction protocol or simply they are the problematic constituents in the sense they show false positive chemical presence or false biological activities. Therefore this article also describes the process to remove out rightly interfering compounds.

**Extraction of secondary metabolites**

Natural products may be obtained from the crushed biological material by extraction with a solvent such as petroleum ether, chloroform ethyl acetate (ethyl ethanoate) or methanol. Several solvents of increasing polarity may be used. Thus lipid material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with non-polar solvents such as petroleum ether, but more polar substances such as the alkaloids (mainly free bases) and glycosides are extracted with methanol, aqueous methanol or even hot water. Many alkaloids are present as their salts with naturally occurring acids such as tartaric acid. Polar solvents dissolve ionic solutes and other polar substances. When it comes to extraction of phytoconstituents, the most widely employed method is extraction using a single solvent at atmospheric pressure which can be boiled owing to their azeotropic nature. Whether the compound(s) to be isolated is chemically undefined or not, it is important to have an idea about the relationship between the method applied and the properties of the substance extracted. A well known and time tested thumb rule is that “like dissolves like”. It means non polar solvents will remove non polar phytoconstituents and vice versa holds equally true. Thus non polar solvents are used to solubilize mostly lipophilic compounds (e.g., alkanes, fatty acids, pigments, waxes, sterols, some terpenoids, alkaloids, and coumarins). Medium-polarity solvents are used to extract compounds of intermediate polarity (e.g., some alkaloids, flavonoids), while more polar ones are used for polar compounds (e.g., flavonoid glycosides, tannins, some alkaloids). Water is not used often as an initial extractant, even if the aim is to extract water-soluble plant constituents (e.g., glycosides, quaternary alkaloids, tannins). There are many methods based on the technique or set up used but this text will explore only classical method for extraction of saponins, because such methods are easy, putative and can be implemented in most of the laboratories in limited setups[4-6].

**Extraction of saponins**

Powdered plant material is extracted by successive solvent treatment in a Soxhlet extractor. First powdered material is defatted with petroleum ether or n hexane. Defatted material is then extracted with methanol. Methanolic extract is concentrated using suitable means (preferably under vacuum by rotary evaporation), producing dry extract. Dried methanolic extract is them suspended into distilled water and shaken with n butanol, followed by precipitation of crude saponins mixture by addition of solvent ether[7,8].

**Estimation of saponins**

20 g of sample is put into a conical flask and 100 ml of 20% aqueous ethanol is added. Above solution is heated on a bather bath for 4 h with continuous stirring at about 55°C. The mixture is filtered and the residue is again extracted with 200 ml 20% ethanol. The combined extracts are concentrated to about 40 ml over water bath at about 90°C. The concentrate is transferred into a 250 ml separating funnel and 20 ml of diethyl ether is added and shaken vigorously. The aqueous layer is recovered while the ether layer is discarded. The purification process is repeated. 60 ml of n-butanol is added. The combined n-butanol extracts are washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution is heated in a water bath. After evaporation the samples is dried in the oven to a constant weight and the saponin content is calculated as percentage[9].

**Thin Layer Chromatography**[10]

Saponins are generally present in plants as glycosides. Powdered drug is extracted by heating under reflux with 70% alcohol. Filtrate is evaporated and concentrated solution is used for TLC. To enrich above concentrated solution in saponins, it is shaken with water saturated-n butanol. Finally organic layer is separated, concentrated to get a fraction rich in saponins. Most commonly employed solvent system are Chloroform- glacial acetic acid-methanol-water (60:32:12:8) and ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26).

**Detecting agents**

Following spraying reagents are used to detect the presence of saponins on TLC plates are: Blood reagent (Hemolytically active saponins are seen as white zones on a reddish background. Hemolysis may be seen instantly

or after drying the plate oven for few second), vanillin-sulphuric acid (saponins show blue, blue-violet, red or yellow-brown zones), anisaldehyde-sulphuric acid (colors are same as depicted by vanillin-sulphuric acid sprayed chromatogram, but exposure to UV-365nm results in blue, violet and green fluorescent zones).

## DISCUSSION

Currently there are a number of well-established methods available for extraction and isolation of natural products from various sources. An appropriate protocol for extraction and isolation can be designed only when the target compound(s) and the overall aim have been decided. It is also helpful to obtain as much information as possible on the chemical and physical nature of the compound(s) to be isolated. For unknown natural products, sometimes it may be necessary to try out pilot extraction and isolation methods to find out the best possible method. At the time of choosing a method, one should be open-minded enough to appreciate and weigh the advantages and disadvantages of all available methods, particularly focusing on their efficiency and, obviously, the total cost involved. Continuous progress in the area of separation technology has increased the variety and variability of the extraction and isolation methods that can be successfully utilized in the extraction and isolation of natural products. For any natural product researcher, it is therefore essential to become familiar with the newer approaches. In most cases, extraction and isolation of natural products are followed by structure determination or confirmation of the purified components. With the introduction of various hyphenated techniques, it is now possible to determine the structure of the compound as separation is carried out, Because of the phenomenal progress made in the area of MS and NMR in the last few decades, it has now become possible to deduce the structure of a compound in microgram amounts, thereby further blurring the boundaries between analytical and preparative methods[11].

## CONCLUSION

Plant secondary metabolites are being studied in various parts of the world to find new molecules, to decipher new cues for drug discovery. Extraction is removal of desired substances from undesired ones. Successful extraction involves selection of right solvent which can extract out maximum quantity of targeted chemicals, while minimizing the interference of unwanted components. To prevent breakdown of important metabolites or artifact formation as a result of extraction conditions or solvent impurities is equally important. Traditional solvent-based procedures are still employed in most of the laboratories despite the fact that they lack reproducibility and are both time-and solvent consuming. This is because they only require basic glasswares and easy.

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