Review Article

Extraction, Estimation and Thin Layer Chromatography of Tannins: A Review

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ABSTRACT

Since time immemorial plant metabolites are in use in various parts of the world for sure shot treatment or for approximate and rudimentary treatment of various ailments. Tannins are water-soluble polyphenols that are present in many plants. They have been reported to be responsible for decrease in feed intake, growth rate, feed efficiency etc. in experimental animals. Till date they are among the most studied phytoconstituents, by scientist of various backgrounds (botanist, biochemist, pharmacognosist, pharmacologist, natural product chemist etc). Various tannins have been reported in literature for range of purposes. Worldwide drug discovery is going through a rough patch. Various models are being discussed, new approaches are emerging, and synthetic drugs are still enjoying good position among available options, nevertheless 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 tannins are being used in treatment of diseases. Important medicinal plants, rich in tannins, are: pale catechu, myrobalan etc. In this review article an attempt has been made to compile basic and crucial information (extraction methods, thin layer chromatography (TLC) and estimation) of tannins. Compiled information is highly concise and widely reported.

Keywords: Tannins, extraction, estimation, TLC, natural products.

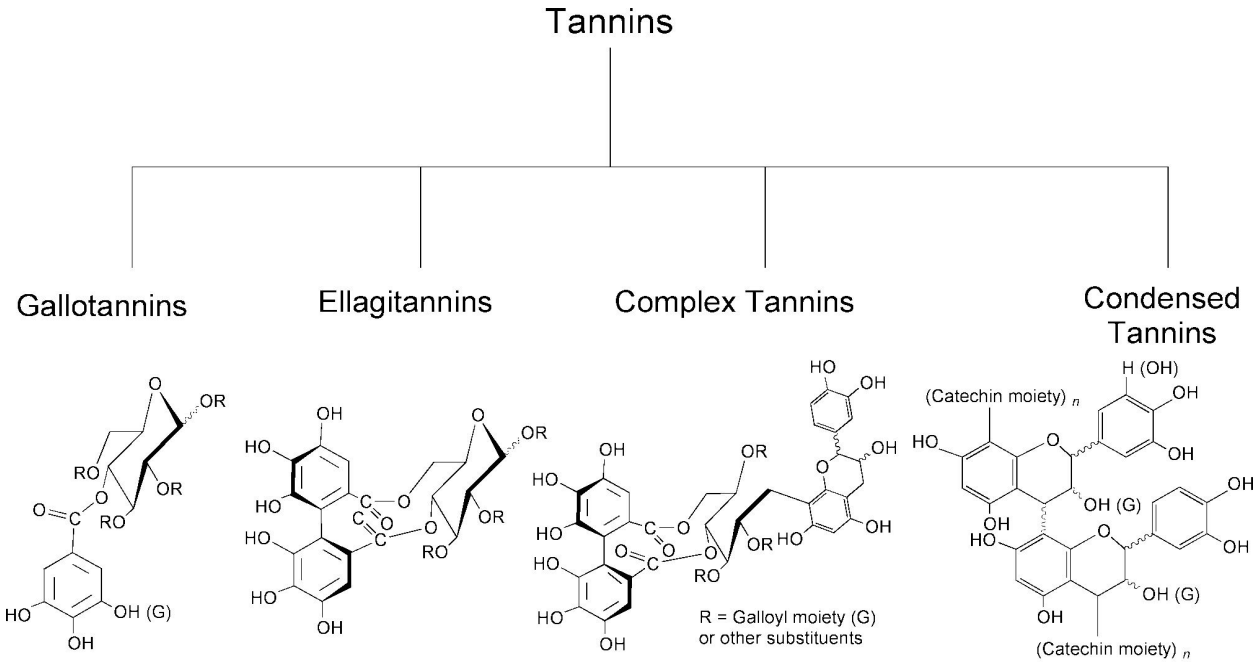
INTRODUCTION

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 select methods for scrupulous and perfect extraction of these metabolites, based on their solubility and other properties. Irrespective of the plant or part thereof or pharmacological activity or subsequent operation, these methods are sufficient to provide perfect extraction of various metabolites viz alkaloids, flavonoids, tannins, saponins, carbohydrates, lignans 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, nonetheless simple extraction using non polar solvents is still enjoy ace poition[1].

In various publications, sometimes extraction schemes are not fully mentioned owing to space constraint or other legitimate motive or some modified process is used. 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]. Present article features the basic research steps with respect to tannins. Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight. It may be noted that all tannins are phenolics but reverse is not true. These are classified into two groups: hydrolysable tannins and condensed tannins[3]. Based on the molecular structures of the currently known tannins, and their origin and role in plant life, the following inclusive and up-to-date definition of the tannins has been written by Karamali K. *et al*[4]. " Tannins are polyphenolic secondary metabolites of higher plants, and are either galloyl esters and their derivatives, in which

galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallotannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins)". Significant quantities of tannins are found in oak galls and these serve as the source of tannic acid. Tannins are used in the treatment of burns as they precipitate the proteins of exposed tissues to from a protective coating[5]. 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 tannins. 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 has more relevance as most of pharmacognostical or phytochemical studies involve removal of tannins as early as possible from the plant material, either because of their inert status or to avoid tannins' interference in subsequent research steps.

Classification of tannins[4]



Extraction of secondary metabolites

Natural products may be obtained from the crushed biological material by extraction with a solvent such as petroleum ether, chloroform (trichloroniethane), ethyl acetate (ethyl ethanoate) or methanol. Several solvents of increasing polarity may be used. Fatty material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with such non-polar solvents as petroleum ether or n hexane, but more polar substances such as the alkaloids (mainly free bases) and glycosides can be removed using methanol, aqueous methanol. 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 almost all the time. Water is usually avoided as an initial extractant, even if the metabolites under question are water-soluble (e.g., glycosides, quaternary alkaloids, tannins). One problem while handling plant fro extraction is the senescent process, in which the cellular integrity is lost and enzymes come in contact with substrates to which they are not normally exposed in living cells. This caused enzymatic action and increase in oxidation process, which is a common problem with tannins (being phenolic) since these are prone to oxidation. Oxidation of phenolics leads to their conversion into quinones, which may be followed by polymerization reaction. In order to avoid these changes, the metabolic activities of the cells need to be checked instantly[6]. There are many methods based on the technique or set up used but this text will explore only classical method for extraction of tannins, because such methods are easy, putative and can be implemented in most of the laboratories in limited setups[6, 7, 8, 9].

Extraction of tannins

Dried plant material is finely powdered. The extract is then prepared by stirring powdered material with 200 ml water for 3 h at room temperature. The crude extract obtained is filtered and concentrated using a rotary evaporator followed by the addition of distilled of water. The extract is loaded on Sephadex LH-20 column to purify the crude tannins mixture using 50% methanol as eluent, followed by elution with 70% acetone. The acetone portion is collected and rotary evaporated under the same conditions[10].

Estimation of tannins

Tannins fall in the category of phenolic compounds. Due to presence of vicinal oxygenated groups, they act as ligands for metal ions. This property is also used to detect tannins. When ferric ions are added to solution of tannins, dark green, blue or blue-black complex is formed[2]. Methods for quantification of tannins may be based on the chemical properties of tannins or their capability to bind substrates, particularly proteins. Here method cited by Paaver U. *et al*, is being reproduced[3]. Total tannins content is determined by using $FeCl_3$ and gelatin tests. 0.1g of the extract is transferred to a 100 ml flask; 50ml of water is added and boiled for 30min. After filtration with cotton filter, filtrate is transferred to a 500ml flask and the volume is made up to the mark with distilled water. 0.5 ml aliquots are transferred to vials, 1 ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ are added and the volume is made up to 10 ml with distilled water. After 5 min absorbance is to be measured at 510 nm against a reagent blank using spectrophotometer and concentration of tannins in the test sample is determined and expressed as mg equivalent of tannic acid per gram of sample[11].

TLC of tannins

Thin layer chromatography is performed using pre-coated silica gel G plates using chloroform-methanol-water (65:35:10) as mobile phase. Chromatogram is developed by spraying plate with 0.5% (w/v) vanillin solution prepared in 4% (w/v) HCl[12].

DISCUSSION

Tannins are characterized by their ability to react with proteins of animal hides and converting them into leather. Another well known feature of tannins is that they react with alkaloids, and therefore it is recommended to use calcium hydroxide as the base (while isolating alkaloids) so that it will complex with tannins and makes subsequent steps easier[8]. Currently there are a number of well-established methods available for extraction and isolation of natural products from various sources. An appropriate procedure for extraction and subsequent fractionation or isolation can be designed only when the target compound(s) and the overall aim have been decided beforehand. It is also helpful to obtain complete information related to compound under question. For a compound (or group of compound), least studied, it may be useful and fruitful to try out pilot extraction and isolation methods to find out the best possible method. Continuous development in phytochemistry has lead to employment of various tools and mechanisms in extraction and analysis of various primary and secondary metabolites. Whatever may be the extraction scheme, removal of tannins is sometimes, the first step in a phytochemistry laboratory, owing to former’ reactive and interfering behavior. Tannins interact nonspecifically with a wide range of enzymes. Tannins crosslink with many proteins and inhibit many biological systems. As mentioned in “Laboratory handbook for the Fractionation of Natural Extracts” tannins may be the only compounds present that are responsible for a reputed biological effects[6,13].

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. This article presents an overview of the process of tannins extraction, with an emphasis on common problems encountered and methods for reducing or eliminating these problems. Highly simple and feasible steps have been mentioned in this paper to perform basic operations. 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 to perform. This article may be informative at least for preliminary phytochemical screening and for those who want to explore a plant from tannins point of view or those who want to remove tannins from biological materials to avoid tannins' interference. Moreover this article may be referred by undergraduate and post graduate students enrolled in pharmaceutical sciences.

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