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Subject: Pharmamacognosy & phytochemistry

Unit: Fourth

Topic: Introduction to secondary metabolites (Tannin)

Tannin

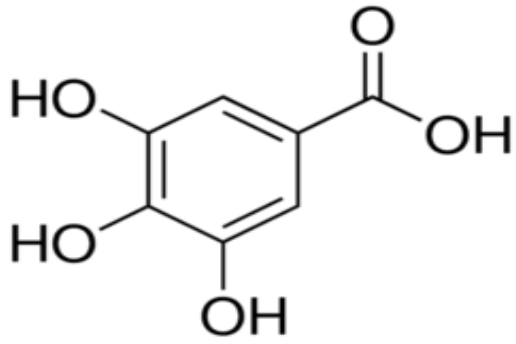
The name **'tannin'** is derived from the French 'tanin'(tanning substance) and is used for a range of naturalpolyphenols. Tannins are complex organic, nonnitrogenous plant products, which generally have astringentproperties. These compounds comprise a large groupof compounds that are widely distributed in the plantkingdom. The term 'tannin' was first used by Seguinin 1796 to denote substances which have the ability tocombine with animal hides to convert them into leatherwhich is known as tanning of the hide. According to this,tannins are substances which are detected by a tanningtest due to its absorption on standard hide powder. Thetest is known as Goldbeater's skin test.

20.2. CLASSIFICATION

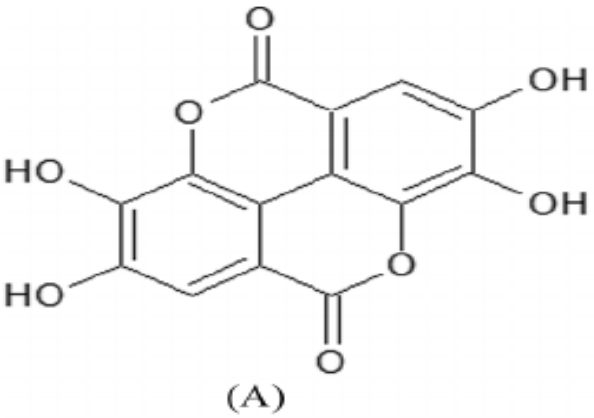
The tannin compounds can be divided into two majorgroups on the basis of Goldbeater's skin test. A group of tannins showing the positive tanning test may be regardedas true tannins, whereas those, which are partly retainedby the hide powder and fail to give the test, are called aspseudotannins.Most of the true tannins are high molecular weightcompounds. These compounds are complex polyphenolicswhich are produced by polymerization of simple polyphenols.They may form complex glycosides or remains assuch which may be observed by their typical hydrolytic reaction with the mineral acids and enzymes. Two majorchemical classes of tannins are usually recognized basedon this hydrolytic reaction and nature of phenolic nuclei involved in the tannins structure. The first class is referredto as hydrolysable tannins, whereas the other class is termedas condensed tannins.

Hydrolysable Tannins

As the name implies, these tannins are hydrolysable bymineral acids or enzymes such as tannase. Their structuresinvolve several molecules of polyphenolic acids such as gallic, hexahydrodiphenic, or ellagic acids, bounded throughester linkages to a central glucose molecule. On the basis of the phenolic acids produced after the hydrolysis, they are further categorized under gallotannins composed of gallicacid or ellagitannins which contains hexahydrodiphenic acid which after intraesterification produces ellagic acid.Hydrolysable tannins are sometimes referred to aspyrogallol tannins as the components of phenolic acids on dry distillation are converted to pyrogallol derivatives.The hydrolysable tannins are soluble in water, and theirsolution produces blue colour with ferric chloride.



Gallic acid



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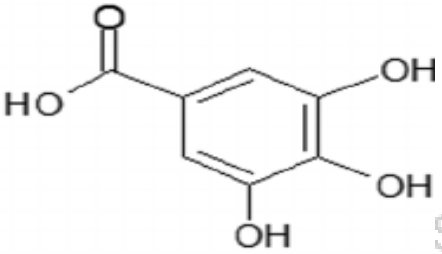

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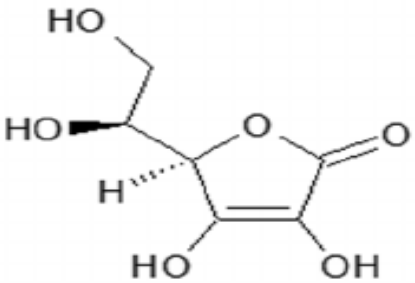

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Ellagic acid & derivative

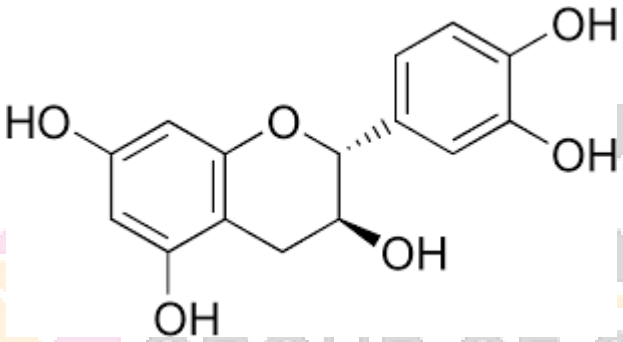
Nonhydrolysable or Condensed Tannins

Condensed tannins, unlike the previously explained groupare not readily hydrolysable to simpler molecules with mineral acids and enzymes, thus they are also referred to as nonhydrolysable tannins. The term proanthocyanidins is sometimes alternatively used for these tannins. The compounds containing condensed tannins contain onlyphenolic nuclei which are biosynthetically related to flavonoids. Catechin which is found in tannins is flavan-3-o1,whereas leucoanthocyanidins are flavan-3,4-diol structures. These phenolics are frequently linked to carbohydrates orprotein molecules to produce more complex tannin

compounds.When treated with acids or enzymes, they tend to polymerize yielding insoluble red coloured products known as phlobaphens. The phlobaphens give characteristic redcolour to many drugs such as cinchona and wild cherry bark. On dry distillation, they yield catechol derivatives.

Condensed tannins are also soluble in water and produces green colour with ferric chloride. The families of the plants rich in both of the abovegroups of tannins include Rosaceae, Geraniaceae, Leguminosae,Combretaceae, Rubiaceae, Polygonaceae, Theaceae,etc. The members of families Cruciferae and Papaveraceae on the other hand are totally devoid of tannins. In the

plants in which tannins are present, they exert an inhibitory effect on many enzymes due to their nature of protein precipitation and therefore contribute a protective function in barks and heartwood.



Catechin

Pseudotannins

Pseudotannins are simple phenolic compounds of lower molecular weight. They do not respond to the tanning reaction of Goldbeater's skin test. Gallic acid, Chlorogenicacid, or the simple phenolics such as catechin are pseudotannins which are abundantly found in plants, especially in

dead tissues and dying cells.

20.3. CHARACTERISTICS OF TANNINS

1. Tannins are colloidal solutions with water.
2. Non crystalline substance.
3. Soluble in water (exception of some high molecular

weight structures), alcohol, dilute alkali, and glycerin.

4. Sparingly soluble in ethyl acetate.
5. Insoluble in organic solvents, except acetone.
6. Molecular weight ranging from 500 to >20,000.

7. Oligomeric compounds with multiple structure units with free phenolic groups.

8. Can bind with proteins and form insoluble or soluble tannin—protein complexes.

20.4. BIOSYNTHESIS OF TANNINS

Tannins belong to the phenolics class of secondary metabolites.All phenolic compounds; either primary or secondary are in one way or another formed through shikirnic acid pathway (phenylpropanoid pathway). Other phenolics such as isoflavones, coumarins, lignins, and aromatic amino acids (tryptophan, phenylalanine, and tyrosine) are also formed by the same pathway. Hydrolysable tannins (Hts) and condensed tannins (proanthocyanidins) are the two main

categories of tannins that impact animal nutrition. Common tannins are formed as follows:

_ Gallic acid is derived from quinic acid._ Ellagotannins are formed from hexahydroxydiphenicacid esters by the oxidative coupling of neighbouring gallic acid units attached to a D-glucose core._ Further oxidative coupling forms the hydrolysable tanninpolymers._ Proanthocyanidin (PA) biosynthetic precursors are the leucocyanidins (flavan-3,4-diol and flavan-4-ol) whichon autoxidation, in the absence of heat, form anthocyanidinand 3-deoxyanthocianidin, which, in turn,polymerize to form PAs.

20.5. CHEMICAL TESTS

1. ***Goldbeater's skin test*:** Goldbeater's skin is a membraneproduced from the intestine of Ox. It behaves justlike untanned animal hide. A piece of goldbeaters skin previously soaked in 2% hydrochloric acid and washed with distilled water is placed in a solution oftannin for 5 minutes. It is then washed with distilledwater and transferred to 1% ferrous sulphate solution.A change of the colour of the goldbeater's skinto brown or black indicates the presence of tannin. Hydrolysable and condensed tannins both give thepositive goldbeater's test, whereas pseudotannins showvery little colour or negative test.

2**. *Phenazone Test*:** To 5 ml of aqueous solution of tannincontaining drug, add 0.5 g of sodium acid phosphate.Warm the solution, cool, and filter. Add 2% phenazone solution to the filtrate. All tannins are precipitated as bulky, coloured precipitate.

3. ***Gelatin Test*:** To a 1% gelatine solution, add little 10% sodium chloride. If a 1% solution of tannin is added to the gelatine solution, tannins cause precipitation of gelatine from solution.

4. ***Test for Catechin* (*Matchstick Test*):** Catechin test is the modification of the well-known phloroglucinol test for lignin. Matchstick contains lignin. Dip a matchstick in the dilute

extract of the drug, dry, moisten it with concentrated hydrochloric acid, and warm it neara flame. Catechin in the presence of acid producesphloroglucinol which stains the lignified wood pinkor red.

5. ***Test for chlorogenic acid***: A dilute solution of chlorogenicacid containing extract, if treated with aqueous ammonia and exposed to air, slowly turns green indicating the presence of chlorogenic acid.

6**. *Vanillin-hydrochloric acid test****:*** Drug shows pink or red colour with a mixture of vanillin: alcohol : dilute HCl in the ratio 1:10:10. The reaction produces phloroglucinol which along with vanillin gives pink or red colour.

20.6. ISOLATION

Both hydrolysable and condensed tannins are highly solublein water and alcohol but insoluble in organic solvents such as solvent ether, chloroform, and benzene. Tannin compounds can be easily extracted by water or alcohol.The general method for the extraction of tannic acid fromvarious galls is either with water-saturated ether, or withmixture of water, alcohol, and ether. In such cases, free acids such as Gallic and ellagic acid go along with ether,whereas true tannin gets extracted in water. If the drugconsists of chlorophyll or pigment, it may be removed byether. After extraction, the aqueous and ethereal layers are separately concentrated, dried, and subjected to further isolation and purification using various separation techniques of chromatography.

20.7. MEDICINAL PROPERTIES AND USES

Tannins occur in crude drugs either as major active constituentas in oak bark, hammamelis leaves, and bearberryleaves, etc. or as a subsidiary component as in clove, cinnamon,peppermint, or garden sage. In many cases, they synergisticallyincrease the effectiveness of active principles.Tannins are medicinally significant due to their astringentproperties. They promote rapid healing and the formationof new tissues on wounds and inflamed mucosa. Tannins are used in the treatment of varicose ulcers, haemorrhoids,minor burns, frostbite, as well as inflammation of gums.Internally tannins are administered in cases of diarrhoea,intestinal catarrh, and in cases of heavy metal poisoning as an antidote. In recent years, these compounds havedemonstrated their antiviral activities for treatment of viraldiseases including AIDS. Tannins are used as mordant indyeing, manufacture of ink, sizing paper and silk, and forprinting fabrics. It is used along with gelatine and albuminfor manufacture of imitation horn and tortoise shell. Theyare widely

used in the leather industry for conversion of hide into leather, the process being known as tanning.Tannins are also used for clarifying beer or wine, in photographyor as a coagulant in rubber manufacture. Tanninsare used for the manufacture of gallic acid and pyrogallol,and sometimes as a reagent in analytical chemistry.