

4.5. TANNINS

4.5.1. Introduction

The term **tannin** was first used by **Seguin** in **1976** to denote substances present in plant extracts which are able to combine with proteins of animal hides, prevent their putrefaction, and convert them into leather. On this basis tannin is a substance which is detected qualitatively by a tanning test (the Goldbeater's skin test) and is determined quantitatively by its absorption on standard hide powder.

Tannins are defined as, "**complex substances that usually occur as mixtures of polyphenols that are very difficult to separate since they do not crystallise**".

It excludes simpler phenolic substances, often present with tannins such as gallic acid, catechins, and chlorogenic acid which may under certain conditions give precipitates with gelatin and be partly retained by hide powder. Tannins are present in the aerial parts, e.g., leaves, fruits, barks, or stem, generally occurs in immature fruits, but disappears during the ripening process. New leaves of deciduous plants contain high concentration of tannins. Tannins occur in many crude drugs. They probably serve as a protection for the plants during growth and are destroyed or deposited as end-products of metabolism in some dead tissues of the mature plants. They are very complex substances, whose isolation in the pure state possesses difficulty. Many types of tannins occur as glycosides.

4.5.2. Occurrence and Distribution

Rhubarb, red rose petals, bearberry leaves, Chinese galls, Turkish gall, hamamelis, chestnut, and maple are rich in **gallitannins**. Pomegranate rind, pomegranate bark, myrobolans, eucalyptus leaves, some Australian kinds and Oak bark are rich in **ellagitannins**.

Some drugs, e.g., tea, hamamelis bark, and hamamelis leaves contain both **hydrolysable** and **condensed tannins**.

Following parts of plants are rich in **condensed tannins**:

- 1) **Barks:** Cinnamon, wild cherry, cinchona, willow, acacia, oak, and hamamelis.
- 2) **Roots and Rhizomes:** Krameria and male fern.
- 3) **Flowers:** Lime and hawthorn.
- 4) **Seeds:** Cocoa, guarana, kola, and areca.
- 5) **Leaves:** Hamamelis, howathron, and tea.
- 6) **Extracts and Dried Juice:** Catechu, acacia, mangrove cutches, East Indian Kino, butea gum, eucalyptus, and kino.

Following are the **examples** which are rich in **pseudotannins**:

- 1) **Gallic Acid:** Rhubarb and most of the materials containing gallitannins.

- 2) **Catechins:** Catechu, acacia cutch, many Australian kinos, cocoa, guarana, and other drugs containing condensed tannins.
- 3) **Chloregenic Acid:** Mate, coffee (particularly unroasted), and nux-vomica (a small quantity only).

Tannins are found abundantly in the plant kingdom. They occur in gymnosperms as well as angiosperms. Tannins in angiosperms are more common in dicotyledons than in monocotyledons.

Examples of families of dicotyledons containing high content of tannins are:

- 1) **Leguminosae:** *Acacia* sp. (wattle), *Sesbania* sp., *Lotus* sp. (trefoil), and *Onobrychis* sp. (sainfoin),
- 2) **Anacardiaceae:** *Scinopsis balansae* (quebracho),
- 3) **Combretaceae:** Myrobalan,
- 4) **Rhizophoraceae:** Mangrove,
- 5) **Myrtaceae:** *Eucalyptus* sp. and *Mirtus* sp. (Myrtle),
- 6) **Polinaceae:** Canaigre.

Some other tannin-containing plants are *Quercus* sp. (oak), *Acer* sp. (maple), *Betula* sp. (birch), *Salix caprea* (willow), *Pinus* sp. (Pine), and *Sorghum* sp.

Tannins are present in **vacuoles** or **surface wax** of the plants; hence, these do not interfere with plant metabolism. When the cells break down and death occurs, tannins can impart metabolic effects.

4.5.3. Isolation

The steps involved in extracting tannins are:

- 1) 100gm of the powdered sample is refluxed for 15 minutes in a 500ml Erlenmeyer flask. During the reflux process, the entire sample should be covered with 70% acetone. The flask is removed from the hot plate and filtered in another flask.
- 2) The residue on the filter paper is washed off using a sufficient amount of 70% acetone.
- 3) The washed solution is combined with the filtrate and the resultant mixture is subjected to extraction using diethyl ether. This process is repeated five times till the diethyl ether and tannin separate out completely as distinct upper and lower layer, respectively.
- 4) The tannin layer is separated via separatory funnel. The tannin so collected is evaporated till a residue, known as the **extracted tannin**, is obtained which is the final product.
- 5) The tannin percentage is calculated with the following formula:

$$\% \text{tannin} = \frac{\text{Wt. of dried tannin}}{\text{Wt. of the powdered sample}} \times 100\%$$
- 6) After extracting the tannin, the aqueous and ethereal layers are concentrated, dried, further isolated, and purified using various chromatography techniques.

4.5.4. Identification Tests

Tannins undergo the following chemical tests:

- 1) **Goldbeater's Skin Test:** A small piece of goldbeater's skin (a membrane prepared from the intestine of an ox) is soaked in 2% hydrochloric acid, rinsed with distilled water, and placed in a solution of tannin for 5 minutes. The skin piece is washed with distilled water and kept in a solution of ferrous sulphate. A brown or black colour appears on the skin due to the presence of tannins.

- 2) **Gelatin Test:** To a solution of tannin (0.5-1%), aqueous solutions of gelatin (1%) and sodium chloride (10%) are added. A white buff-coloured precipitate is formed.
- 3) **Phenazone Test:** A mixture of aqueous extract (5ml) of a drug and sodium acid phosphate (0.5gm) is heated, cooled, and filtered. A solution of phenazone (2%) is added to the filtrate. A bulky coloured precipitate is formed.
- 4) **Catechin Test (Matchstick Test):** A matchstick is dipped in aqueous plant extract, dried near burner, and moistened with concentrated hydrochloric acid. On warming near a flame, the matchstick wood turns pink or red due to the formation of phloroglucinol.
- 5) **Chlorogenic Acid Test:** An extract of chlorogenic acid containing the drug is treated with aqueous ammonia. A green colour is formed on exposure to air.
- 6) **Vanillin-Hydrochloric Acid Test:** 1gm drug is treated with vanillin-hydrochloric acid reagent. A pink or red colour is formed due to the formation of phloroglucinol.

4.5.5. Therapeutic Effects

The medicinal uses of tannins are:

- 1) **Styptics:** Tannin-containing drugs precipitate proteins, and have been used traditionally as styptics and internally for the protection of inflamed surface of mouth and throat.
- 2) **Anti-Diarrhoeal:** They act as anti-diarrhoeal and have been employed as antidotes in poisoning by heavy metals, alkaloids, and glycosides. In Western medicine, their use declined after World War-II when it was found that absorbed tannic acid can cause severe central necrosis of the liver.
- 3) **Anti-Tumour:** Ellagitannins monomer units having galloyl groups at the C-2 and C-3 positions on the glucose core exhibit a strong anti-tumour activity.
- 4) **Antidote:** They are used as an antidote in alkaloid poisoning. They form insoluble tannates with alkaloids which are non-toxic. Only dilute solution is applied for this purpose.

4.5.6. Pharmaceutical Applications

There are a number of tannins which are used in the pharmaceuticals. Table 4.8 shows the various tannins which are used in the pharmacy:

Table 4.8: Tannins Containing Natural Drugs

Name of Drug and Synonyms	Biological Sources	Active Constituents	Uses
1) Hydrolysable Tannins			
i) Myrobalan (Harde)	Dried mature fruits of <i>Terminalia chebula</i> (Combretaceae)	Chebolic acid, chebulagic acid, and gallic acid.	Astringent, stomachic, purgative, and ingredient of Triphala Churna.
ii) Bahera	Dried ripe fruits of <i>Terminalia bellerica</i> (Combretaceae)	Gallic acid and chebulagic acid	Astringent and ingredient of Triphala Churna.
iii) Arjuna	Dried stem bark of <i>Terminalia arjuna</i> (Combretaceae)	Ellagic acid and β -sitosterol	Cardiotonic and hypotensive.
iv) Tannic Acid	Fermented oak galls which are grown on young twigs of <i>Quercus infectoria</i> (Fagaceae)	Gallic acid and glucose	Astringent for mucous membrane.

v) Amla (Indian Goose Berry)	Dried and fresh fruits of <i>Emblica officinalis</i> (Euphorbiaceae)	Vitamin C and phyllembin, tannins	Diuretic, laxative, and ingredient of Triphala and Chyavanprash.
2) Condensed Tannins			
i) Ashoka Bark	Dried stem bark of <i>Saraca indica</i> (Leguminosae)	Catechol and ketosterol	Uterine tonic and oxytocic.
ii) Black Catechu (Cutch)	Dried aqueous extract of heartwood of <i>Acacia catechu</i> (Leguminosae)	Acacatechin and quercetin	Astringent for boils, skin eruptions, and mainly used for commercial purposes.
iii) Pale Catechu (Gambier)	Dried aqueous extract of leaves and young shoots of <i>Uncaria gambier</i> (Rubiaceae)	Catechin and catechutannic acid	Astringent for treatment of diarrhoea.
iv) Pterocarpus	Dried juice of the plant <i>Pterocarpus marsupium</i> (Leguminosae)	Kinotannic acid, Kinored, and k-pyrocatechin	Hypoglycaemic and powerful astringent.