Tannins.Medicinal plants and raw materials containing tannins.

Tannins (tannides) are complex mixtures of plant high molecular weight polymers of phenolic compounds with molecular weights between 300 and 5000 (on the order of 500-3000), possessing an astringent flavour, capable of forming strong bonds with proteins, turning unworked animal hide into tanned leather.

The essence of the tanning process is the formation of strong hydrogen bonds between phenolic hydroxyls of tannins and collagen protein molecules. The result is a strong cross-linked structure - leather that is resistant to heat, moisture, microorganisms and enzymes, i.e. it cannot rot.

Polyphenolic compounds with lower molecular weight (less than 300) are only adsorbed on proteins, but are not able to form stable complexes, and are not used as tanning agents. High molecular weight polyphenols (with molecular weight more than 5000) are also not tannins because their molecules are too large and do not penetrate between collagen fibrils.Thus, the main difference between tannins and other polyphenolic compounds is their ability to form strong hydrogen bonds with proteins.

Distribution in the plant kingdom

Tannins are widely distributed in nature. They are found mainly in plants, they are also found in algae, fungi and lichens. The most common tannins are found in dicotyledons, where they accumulate in maximum amounts. Dicotyledons usually do not contain tannins, ferns contain tannins, while horsetails, mosses, and plaunas have practically none or minimal amounts. The families with the highest tannin content are: *Anacardiaceae* (Rhus coriaria, Cotinus coggigria); *Rosaceae* (Sanguisorba officinalis, Potentilla erecta); *Fagaceae* (Quercus robur); *Polygonaceae* (Polygonum bistorta); *Ericaceae* (bearberry, cowberry); Betulaceae (Alnus incana, Alnus glutinosa) and others. The content of tannides in plants reaches 20-30 %, the highest content of tannins is found in pathological formations - galls (up to 50-70 %).

Role for plants

The biological role for plant life is not fully elucidated. There are several hypotheses:

1. tannins are waste products of plant organisms;
2. tannins are one of the forms of spare nutrients. This is indicated by their localisation in underground organs and bark;
3. tannins fulfil a protective function, because when plants are damaged, they form complexes with proteins, which create a protective film preventing the penetration of phytopathogenic organisms. They have bactericidal and fungicidal properties;
4. tannins are involved in redox processes, are oxygen carriers in plants.

Classification of tannins

Since tannins are mixtures of different polyphenols with diverse chemical compositions, their classification is difficult.For example, they were previously classified according to the nature of their decomposition products at a temperature of 180-200o C without access to air. According to this classification, they are divided into 2 groups:

1. Pyrogallol - give pyrogallol at decomposition
2. Pyrocatechin - pyrocatechin is formed

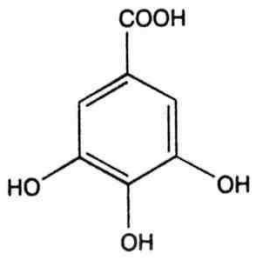
The classification of G. Povarnin (1911) and K. Freidenberg (1933), based on the chemical nature of tannins and their relation to hydrolysing agents, is most recognised. According to this classification, tannins are divided into two broad groups:

-hydrolysable tannides;

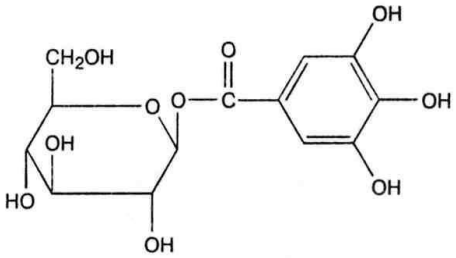
-condensed tannides.

1. ***Hydrolysable tannins*** are mixtures of esters of phenolcarboxylic acids with sugars and non-saccharides. In aqueous solutions under the action of acids, alkalis and enzymes, they can be hydrolysed into phenolic and non-phenolic constituents. Hydrolysable tannins can be divided into three groups.

1.1. *Gallotannins* are esters of gallic acid, digallic acid and its other polymers with cyclic forms of sugars (usually D-glucose).

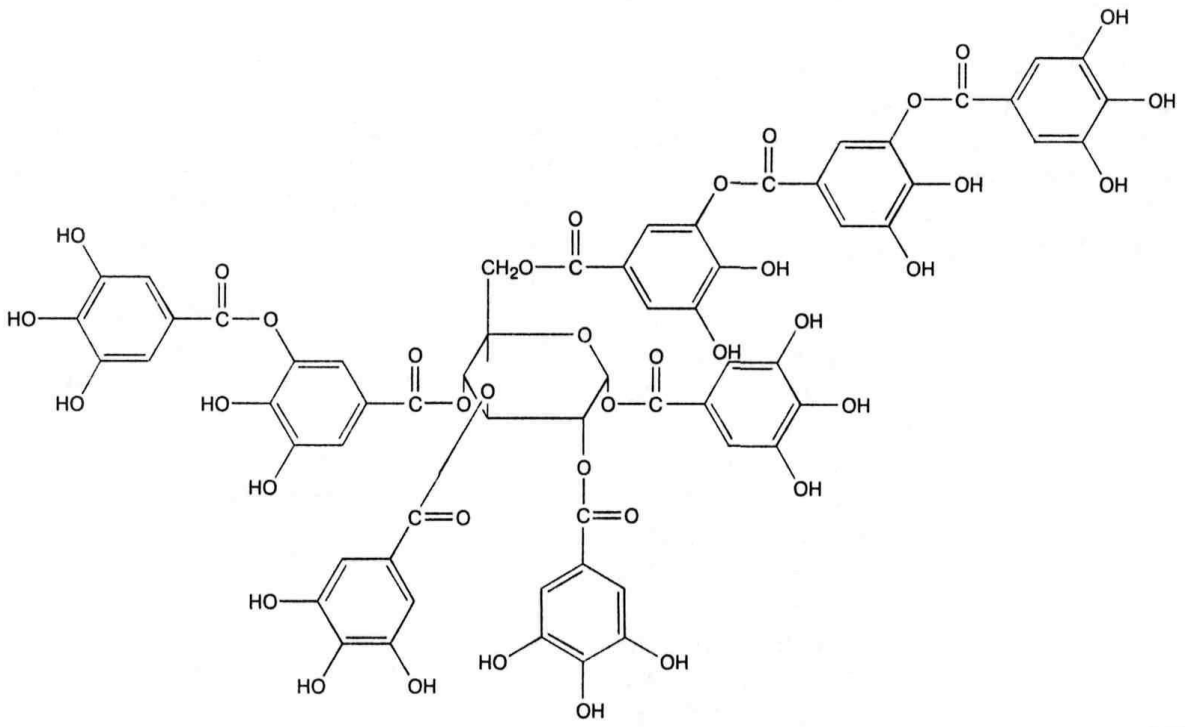


Gallic acid



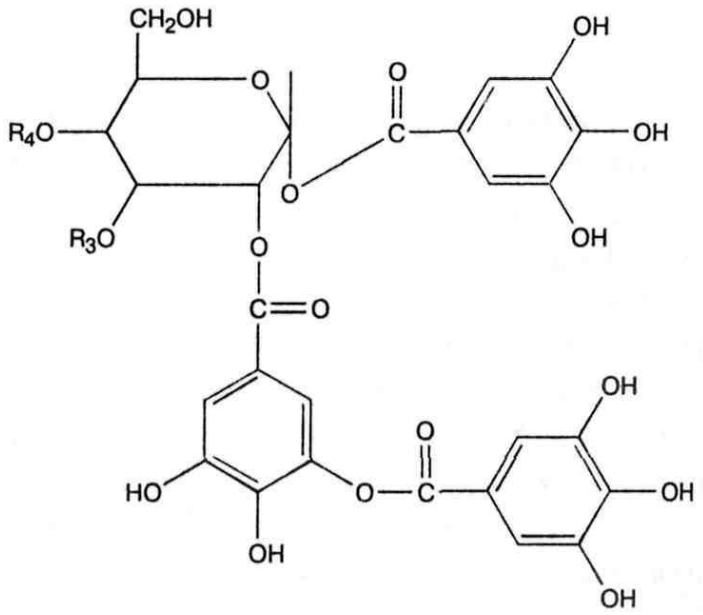
β-Glucogallin

Industrial sources of gallotannins used in medicine (medical tannin) are Turkish galls - pathological growths formed on dyer's oak (Quercus infectoria Oliv.), Chinese galls formed on Chinese sumac (Rhus chinensis Mill.), leaves of sumac tannin (Rhus coriaria L.) and leaves of tannery scumpia (Cotinus coggygria Scop.). Tannin is a heterogeneous mixture of substances of different structures. Mono-, di-, tri-, tetra-, penta- and polyhalloyl ethers occur.A detailed deciphering of the structure of tannin was given in 1961-1963 by W. Haworth. Chinese tannin isolated from Chinese galls is octa- and nonagalloylglucose.



The structure of Chinese tannin

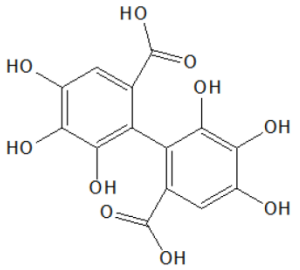
Turkish tannin, isolated from Turkish galls, is a hexa- and heptagalloylglucose.



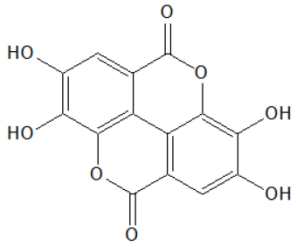
Structure of Turkish tannin
(R_3 = gallic acid; R_4 = m-digallic acid)

Tannins of this group are contained and predominate in rhizomes and roots of *Sanguisorba officinalis*, rhizomes of *Polygonum bistorta*, rhizomes of *Bergenia crassifolia*, fruits of *Alnus incana*, bark of *Quercus robur*.

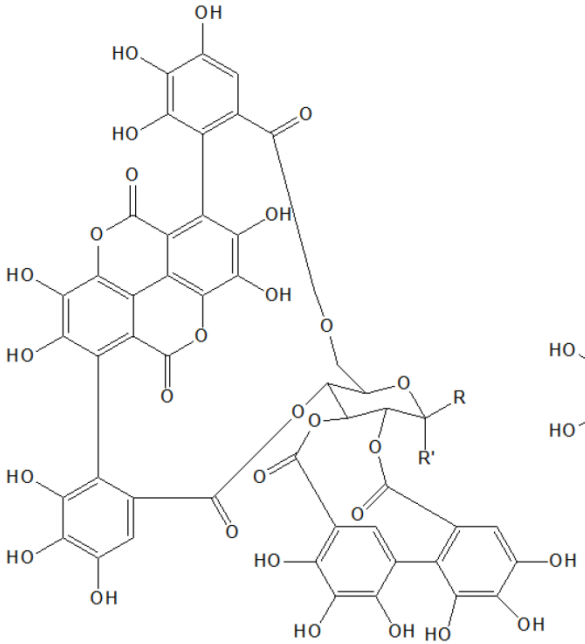
1.2. *Ellagotannins* are esters of ellagic acid and other acids biogenetically related to it with cyclic forms of sugars (D-glucose).



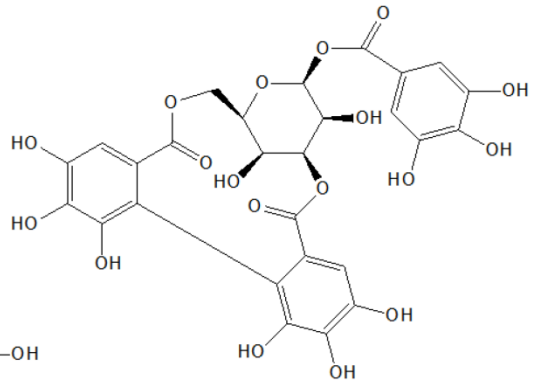
Hexahydroxydiphenic acid (HHDP)



Ellagic acid



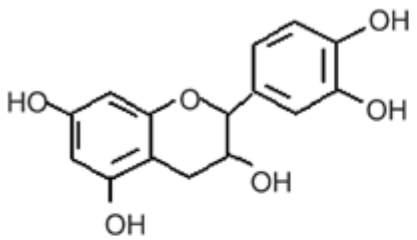
alpha-Punicalagin (R=H, R′=OH)
beta-Punicalagin (R=OH, R′=H)



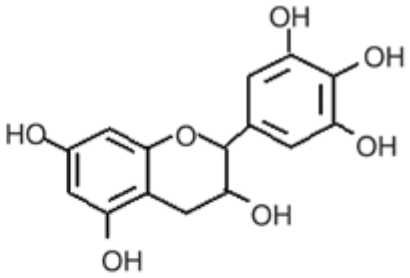
Corilagin

Ellagotannins are complex in structure and are found mainly in tropical and subtropical plants. They are found in the pericarp of pomegranate fruit (*Punica granatum*), eucalyptus bark, walnut pericarp (*Juglans regia*), oak bark, leaves and inflorescences of *Epilobium angustifolium* (willow weed).Gallotannins and ellagotannins can occur simultaneously in plants.

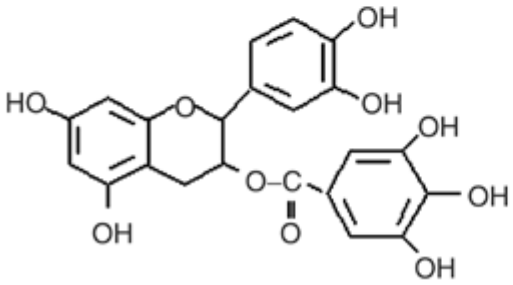
1.3. *Non-saccharide esters of phenolcarboxylic acids* are esters of gallic acid with cinnamic acids, hydroxycinnamic acids (chlorogenic acid, caffeic acid, hydroxycinnamic acid), and flavans (catechin gallate).This group is widely distributed in plants. Gallic acid esters and catechins are found in the leaves of Chinese tea - Camellia sinensis (L.) Kuntze. Theogallin, which is an ester of cinnamic and gallic acids (3-O-galloylquinic acid), has been isolated from green tea.



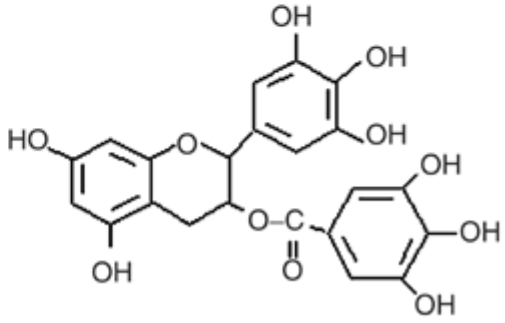
(–)–Epicatechin (EC)



(–)–Epigallocatechin (EGC)

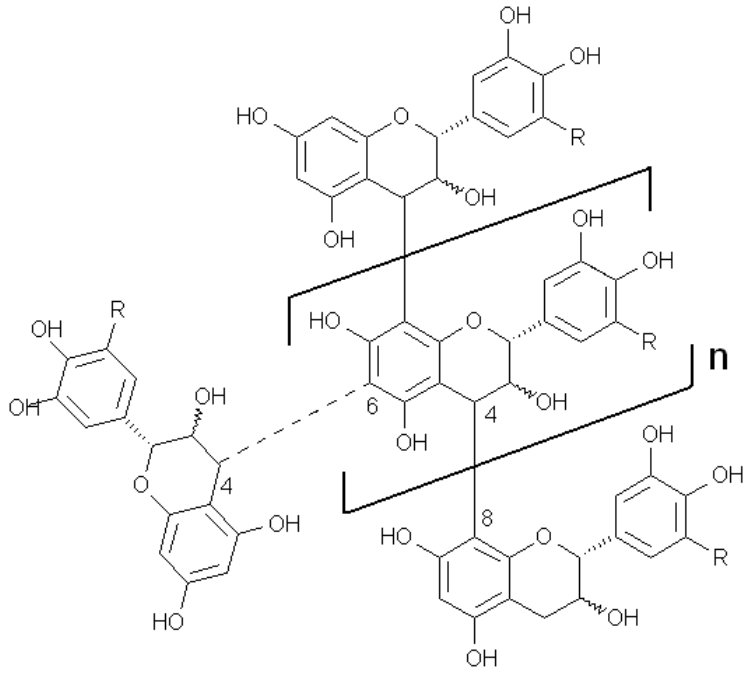


(–)–Epicatechin-3-gallate (ECG)



(–)–Epigallocatechin-3-gallate (EGCG)

2. ***Condensed tannins*** do not have the character of esters, the polymer chain of these compounds is formed by means of carbon-carbon bonds (-C-C-), which makes them resistant to acids, alkalis and enzymes. Under the action of mineral acids, they do not split, but increase in molecular mass with the formation of oxidative condensation products - phlobaphenes, or reds, red-brown in colour.Condensed tannins are condensation products of catechins (flavan-3-ols), leucoanthocyanidins (flavan-3,4-diols), and less frequently hydroxystilbenes (phenylethylenes).



The formation of condensed tannins can proceed in two ways. According to K. Freidenberg, it is accompanied by the rupture of the pyran ring of catechins, and the C2-atom of one molecule is joined by a carbon-carbon bond to the C6- or C8-atom of another molecule.

According to D. E. Hathaway, condensed tannins are formed as a result of enzymatic oxidative condensation of catechin and leucoanthocyanidin molecules in a head-to-tail (ring A to ring B) or tail-to-tail (ring B to ring B) pattern according to the positions 5'→8; 5'→2'' and others.

Condensed tannins are contained and predominate in the rhizomes of tormentilla (*Potentilla erecta*), blueberry fruit, bird cherry fruit (*Padus avium*), St John's wort herb, and tea leaves.

Mixtures of tannins also include simple phenols (resorcinol, pyrocatechin, pyrogallol, floroglucin, etc.) and free phenolcarboxylic acids (gallic, ellagic, protocatechic, etc.).

Most often in plants there is a mixture of hydrolysable and condensed tannides with predominance of one or the other group, therefore it is rather difficult to classify medicinal plant raw materials by type of tannins.

In some raw materials almost equal content of both groups of tannins is observed (e.g., rhizomes of snake root (bistort)).

Biosynthesis, localisation and accumulation of tannins in plants

The biosynthesis of hydrolysable tannins follows the shikimate pathway, while condensed tannins are formed by a mixed pathway (shikimate and acetate-malonate).Tannins are dissolved in the vacuoles of plant cells and adsorbed on cell walls during cell aging.
They are localised in epidermal cells, sheathing cells surrounding conductive bundles (leaf veins), in parenchyma cells of medullary rays, bark, wood and phloem.
Tannins are accumulated in large quantities mainly in underground organs of perennial herbaceous plants (rhizomes of Bergenia crassifolia, Polygonum bistorta, Potentilla erecta, rhizomes and roots of Sanguisorba officinalis), in bark and wood of trees and shrubs (bark of Quercus), in fruits (fruits of Padus avium, Alnus), less frequently in leaves (leaves of Cotinus coggigria, sumac, tea).

The accumulation of tannides depends on genetic factors, climatic and environmental conditions. In herbaceous plants, as a rule, the minimum amount of tannins is observed in spring during shoot regrowth, then their content increases and reaches the maximum during budding and flowering (e.g., rhizomes of Potentilla erecta).

Towards the end of vegetation, the amount of tannins gradually decreases. In Sanguisorba officinalis, the maximum amount of tannins is accumulated during the phase of rosette leaf development, decreases during the flowering phase, and increases again in autumn. The vegetation phase affects not only the quantity but also the qualitative composition of tannins. In spring, during the period of sap movement in the bark of trees and shrubs and in the phase of shoot regrowth in herbaceous plants, hydrolysable tannides are predominantly accumulated, and in autumn, during the phase of plant death - condensed tannides and products of their polymerisation - phlobaphenes (redsenes).

The most favourable conditions for accumulation of tannides are temperate climate conditions (forest zone and high alpine belt).The highest content of tannins is observed in plants growing on dense calcareous soils, their content is lower on loose chernozem and sandy soils. Phosphorus-rich soils contribute to the accumulation of tannins, while nitrogen-rich soils reduce tannid content.

Harvesting, drying and storage of raw materials containing tannins

Harvesting of medicinal plant raw materials containing tannins is carried out according to the general rules. However, there are some exceptions to the rules:

-Tormentilla (Potentilla erecta) rhizomes are harvested in summer, during flowering, because the content of condensed tannins in them is quite high, and also take into account the fact that after flowering of the plant and fading of its above-ground part, in autumn, it is almost impossible to find lapchatka in the herbage of boggy areas;

-the rhizomes of Polygonum bistorta are dug up immediately after the plant has flowered;

-rhizomes and roots of Sanguisorba officinalis be dug up during the fruiting period, when the dark red inflorescences are easily visible in the grass;

-fruits of Alnus incana and Alnus glutinisa are collected in late autumn or winter, when the leaves are not interfering.

They are dried in dryers at temperatures not exceeding 60 ºC (40-60 ºC). In natural drying, the raw material is spread out in a thin layer in the open air or in a closed, ventilated room. Raw materials can be dried in the sun, as tannins do not decompose under the influence of ultraviolet rays.

Store raw materials containing tannins, should be stored according to the general rules. Fruits of Padus avium and bilberry are stored separately, together with other fruits. Fruits of Alnus are stored together with all types of raw materials, because saplings are woody and, as experience has shown, are not subject to spoilage by barn pests.

Physical and chemical properties

Tannins are extracted from plant raw materials in the form of a mixture of polymers and are amorphous substances of yellow or yellow-brown colour, odourless, astringent, very hygroscopic. They are well soluble in water (especially in hot water) with formation of colloidal solutions; they are also soluble in ethyl and methyl alcohols, acetone, ethyl acetate, butanol, pyridine. Insoluble in chloroform, benzene, diethyl ether and other non-polar solvents, optically active. Easily oxidised in air.

They are able to form strong intermolecular bonds with proteins and other polymers (pectin substances, cellulose, etc.). Under the action of enzymes and acids hydrolysable tannins are broken down into their constituent parts, condensed tannins are polymerised.

They are precipitated from aqueous solutions by gelatin, alkaloids, lead basic acetate, potassium bichromate, cardiotonic glycosides.

As substances of phenolic nature, tannins are easily oxidised by potassium permanganate in acidic medium and other oxidants, form coloured complexes with salts of heavy metals, trivalent iron, bromine water. They are easily adsorbed on skin powder, cellulose, cotton wool.

Analysis of raw materials containing tannins

To obtain the sum of tannins, plant raw materials are extracted with hot water in a ratio of 1:30 or 1:10.

Qualitative analysis. Qualitative reactions (precipitation and colour) and chromatographic study are used.I. General precipitation reactions - for the detection of tannins in raw materials:

1. Specific reaction is the gelatin precipitation reaction, using 1% gelatin solution in 10% sodium chloride solution. A flake-like precipitate or muddy appears, disappearing when excess gelatin is added. A negative reaction with gelatin indicates the absence of tannins.
2. Reaction with salts of alkaloids, 1 % quinine chloride solution is used. An amorphous precipitate appears due to the formation of hydrogen bonds between the hydroxyl groups of the tannins and the nitrogen atoms of the alkaloid. These reactions have the same effect irrespective of the tannid group. A number of reactions make it possible to determine whether tannins belong to a particular group.

II. *Group qualitative reactions for tannins* :

№	Reagent	Hydrolysable tannins	Condensed tannins
1	dilute sulphuric acid	hydrolysis	red-brown phlobaphenes
2	bromine water (5g bromine in water)	--------	orange or yellow precipitate
3	1 % solution of iron-ammonium alum (iron oxide chloride is not used, as its solution has an acidic reaction of the medium).	black and blue staining or precipitate	black-green staining or precipitate
4	10 % solution of lead medium acetate (at the same time add 10 % solution of acetic acid)	white precipitate insoluble in acetic acid (the precipitate is filtered and the content of condensed tannides is determined in the filtrate, with 1% solution of ferric alum - black-green colouring).	white precipitate soluble in acetic acid
5	Stiasni test (40% formaldehyde solution with concentrated hydrochloric acid)	---------	brick-red precipitate (filter the precipitate and determine the content of hydrolysable tannides in the filtrate, in neutral medium with 1% ferric alum solution - black-blue staining)
6	1 % solution of vanillin in concentrated hydrochloric acid	---------	orange-red staining (catechins)

Reaction with 1% alcoholic solution of ferric alum is included in all regulatory documents for medicinal raw materials as a reaction to determine their authenticity. The reaction is recommended by the State Pharmacopoeia of Russia XIV and is performed both with decoction of raw materials (oak bark, serpentine rhizomes, alder saplings, bilberry fruits), and for the discovery of tannins directly in dry raw materials (oak bark, calamus bark, rhizomes of badanus).

Quantitative determination.

About 100 different methods are known for the quantitative determination of tannins, which can be divided into the following main groups.

*1. Gravimetri*c, or weight methods - based on quantitative precipitation of tannins by gelatin, heavy metal ions or adsorption by skin (golium) powder.For technical purposes, the gravimetric method using golium powder - the weight unified method (WEM) - is the standard method worldwide.The aqueous extract of tannins is divided into two equal parts. One part of the extract is evaporated and dried to constant weight. The other part of the extract is treated with skin powder and filtered. The tannins are adsorbed on the skin powder and remain on the filter. The filtrate and wash water are evaporated and dried to constant weight. The tannin content is calculated from the difference in weight of the dry residues.The method is inaccurate because skin powder adsorbs low molecular weight phenolic compounds, is rather labour-intensive and expensive.

2. Titrimetric methods. These include:

(a) Gelatin method - based on the ability of tannins to form insoluble complexes with proteins. Aqueous extracts from raw materials are titrated with 1% gelatin solution, at the equivalence point gelatinotannate complexes dissolve in excess of the reagent. The titre is established by pure tannin. The equivalence point is determined by taking the smallest volume of titrated solution that causes complete precipitation of tannins.The method is the most accurate, because it allows you to determine the amount of true tannins. Disadvantages: duration of determination and difficulty in establishing the equivalence point.

*(b) Permanganatometric method (*Leventhal-Neybauer method modified by A.L. Kursanov). This is a pharmacopoeial method, based on the easy oxidation of tannins potassium permanganate in an acidic environment in the presence of an indicator and catalyst indigo sulfonic acid, which at the equivalence point passes into isatin, and the colour of the solution changes from blue to golden-yellow. Features of the determination, allowing to titrate only macromolecules of tannins: titration is carried out in highly dilute solutions (extraction is diluted 20 times) at room temperature in an acidic medium, potassium permanganate is added slowly, drop by drop, with vigorous stirring.The method is economical, fast, easy to perform, but not accurate enough, because potassium permanganate oxidises partially and low molecular weight phenolic compounds.

3. Physico-chemical methods.

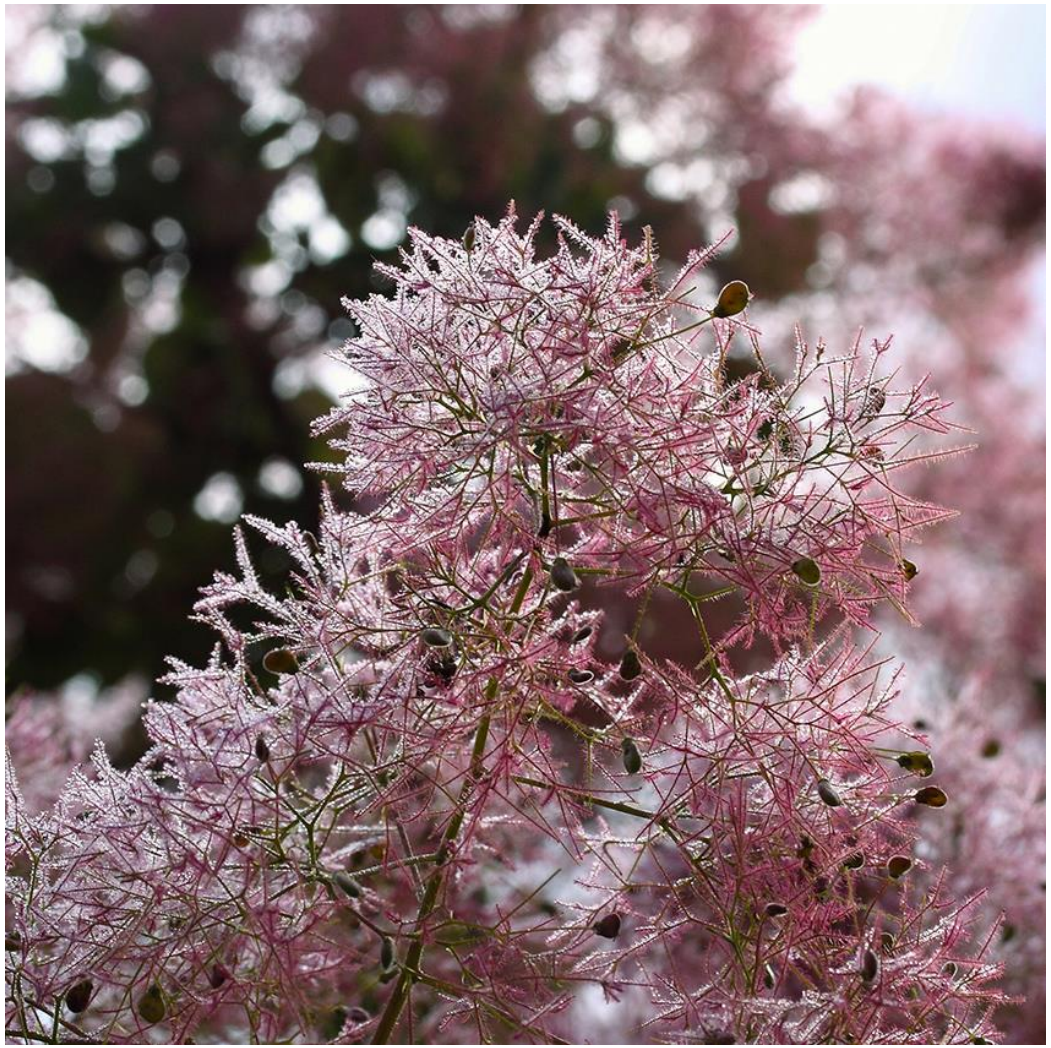
a) Photoelectrocolorimetric methods are based on the ability of tannins to form coloured compounds with trivalent iron salts, phosphoric-tungstic acid, Folin-Denis reagent and others.
b) Chromatospectrophotometric and nephelometric methods are used in scientific research.

Common smoke tree - Cotinus coggygria Scop. (Rhus cotinus L).

Family Anacardiaceae

Common smoke tree leaves - Cotini coggygriae folia

It is a multiple-branching deciduous shrub growing to 5–7 metres tall with an open, spreading, irregular habit, only rarely forming a small tree. The leaves are 3–8 centimetres long rounded ovals, green with a waxy glaucous sheen. The autumn colour can be strikingly varied, from peach and yellow to scarlet. The flowers are numerous, produced in large inflorescences 15–30 cm long; each flower 5–10 millimetres in diameter, with five pale yellow petals.



Most of the flowers in each inflorescence abort, elongating into yellowish-pink to pinkish-purple feathery plumes (when viewed en masse these have a wispy 'smoke-like' appearance, hence the common name "smoke tree") which surround the small drupaceous fruit that develop.

The species is native to a large area from southern Europe, east across central Asia and the Himalayas to northern China. It is commonly grown as an ornamental plant, with several cultivars available. Many of these have been selected for purple foliage and flowers.

Chemical composition.

Common smoke tree leaves contain up to 25% tannins, 3-5% free gallic acid, with young leaves containing more tannins. In addition, the leaves contain flavonoids, essential oil of pleasant odour, the main part of which is myrcene. Scumpia tannin is similar in structure to tannin of Chinese galls.

In raw materials the content of tannin, determined by titration with trilon B, should not be less than 15%.

It is harvested from the beginning of flowering to the full ripening of the fruit by tearing off the whole leaves, which are not damaged by insects. They can be harvested every year from the same thickets. In order to preserve the thickets, the branches must not be broken off.Collected raw materials are dried in well-ventilated rooms. In good weather - in the sun. In artificial drying, the temperature should not exceed 60°C.
The quality of raw materials is regulated by GOST 4564-79.
Stored in a dry place, protected from light, shelf life 2 years.

Uses.

Scumpia leaves serve as a domestic raw material for obtaining medical and technical tannin. Medical tannin has astringent, anti-inflammatory and antiseptic action. Tannin is used for preparation of "Tanalbin" and "Tansal".

From the leaves, the preparation "Flacumin" is obtained, which is the sum of flavonol aglycones isolated from the leaves of scumpia. Flacumin hascholeretic effect and is used in diseases of the liver and biliary tract, especially in their dyskinesia.

tanner's sumach - Rhus coriaria L.
Family Anacardiaceae
tanner's sumach leaves - Rhus coriaria Rhus coriaria folia

Rhus coriaria Linn is commonly known as Sumaq and the leaves have long been well known in Europe and in the East.

R. coriaria is a 1-3 meter heigh shrub or small tree. The leaves are large and imparipinnate with 9-15 leaflets.

The inflorescence is a compact and erect panicle, the flowers are small and greenish white.

The fruits are a small flattened drupe the size of the lentil of red colour, containing one lenticular polished brown seed

The plant is globally distributed in temperate and tropical regions and can grow on marginal lands. The plants have shallow spreading root system that prevent soil erosion and can grow on poor eroded soil. Most common sumac grown commercially on global scale is R. coriaria in Mediterranean and Middle East, having been cultivated for several centuries to produce a material of high quality for tanning. It is found growing naturally in region of Mediterranean, South east and central and northern regions of Turkey

Chemical composition.
Sumac tannin leaves contain 23 - 25% tannin, which is accompanied by free gallic acid and its methyl ester. Also present in large quantities are flavonoid glycosides, derivatives of quercetin, kaempferol, myricetin.

The quality of raw materials is regulated by GOST 4565-79, according to which the content of tannin determined by titrimetric (with trilon B) in the presence of xylenol orange should be at least 15%.

The raw material is harvested in summer by cutting or tearing off the leaves; it is also possible to cut off the young leafy shoots whole. Branches should not be broken off. According to some reports, it can be harvested from the budding phase to full fruit ripening, i.e. from June to September-October.
The thicket can be exploited no more than once every 2 years.
Raw material is dried in the sun, in dryers or under sheds. Shelf life is 2 years.

Pharmacotherapeutic group. Astringent, tannic agent.

Raw material for production of tannin.

Pharmacological properties. Tannin has astringent, anti-inflammatory and antiseptic properties.

Application. Industrial source of medical tannin (similar to common smoke tree leaves).


THANK YOU
FOR YOUR
ATTENTION