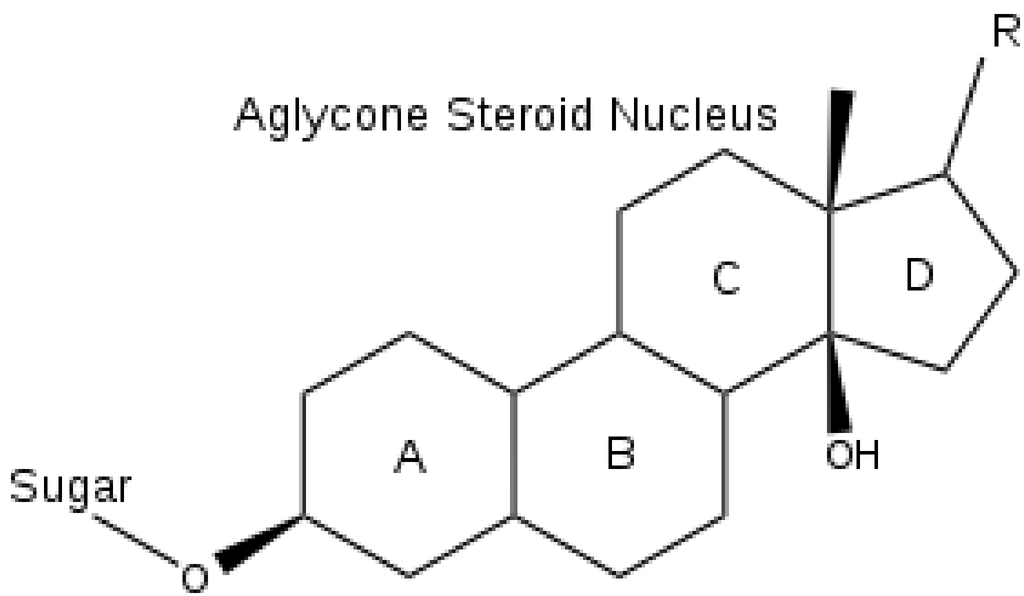
Cardiac glycosides. Classification, methods of analysis. Medicinal plants and raw materials containing cardiac glycosides.

The **cardiac glycosides** are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Plants containing cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C. Throughout history these plants or their extracts have been variously used as arrow poisons, emetics, diuretics and heart tonics. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation and flutter. Yet their toxicity remains a serious problem. These drugs all act by affecting the availability of intracellular Ca+2 for myocardial contraction or increasing the sensitivity of myocardial contractile proteins.

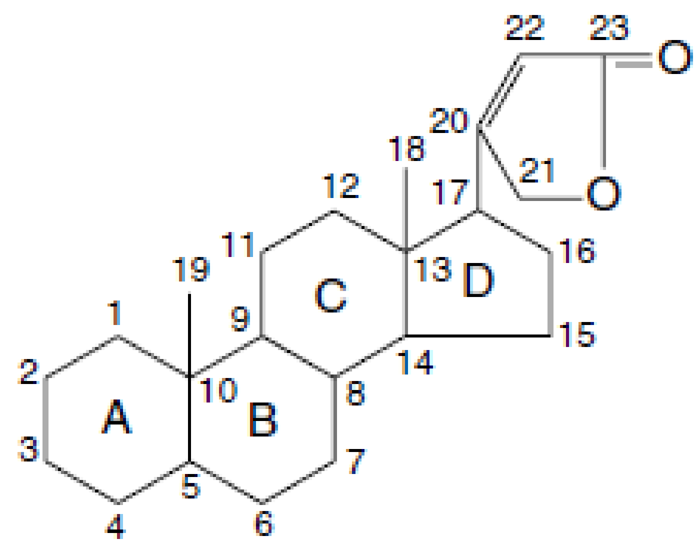
The heart-arresting properties of these glycosides also render them most effective as arrow poisons and a number of tropical plants are better-known in this respect than for their medicinal use.

Cardiac glycosides are composed of two structural features:
the sugar (glycone) and the nonsugar (aglycone–steroid) moieties.

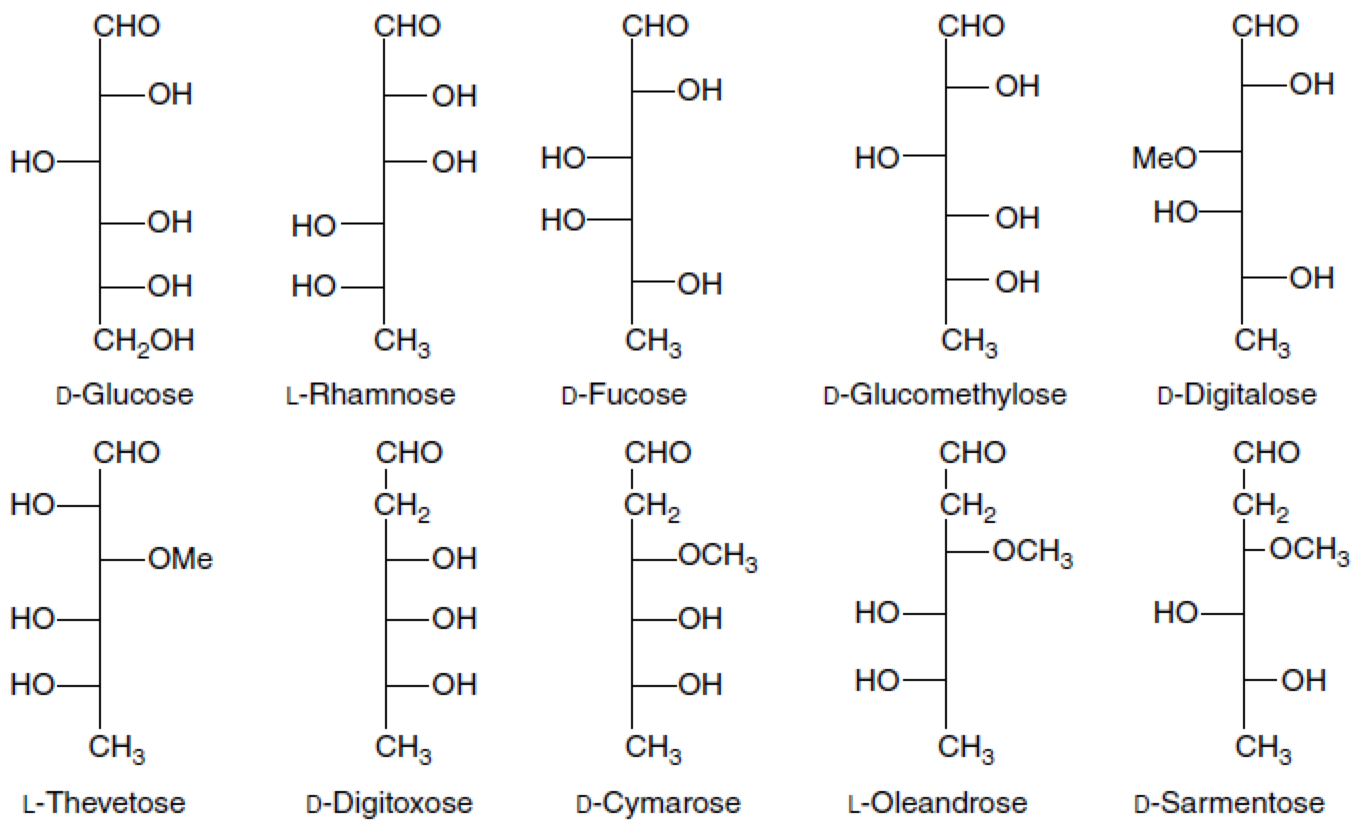


The steroid nucleus has a unique set of fused ring system that makes the aglycone moiety structurally distinct from the other more common steroid ring systems. The steroid nucleus has hydroxyls at 3- and 14-positions of which the sugar attachment uses the 3-OH group. 14-OH is normally unsubstituted. Many genins have OH groups at 12- and 16-positions. These additional hydroxyl groups influence the partitioning of the cardiac glycosides into the aqueous media and greatly affect the duration of action. The lactone moiety at C-17 position is an important structural feature.

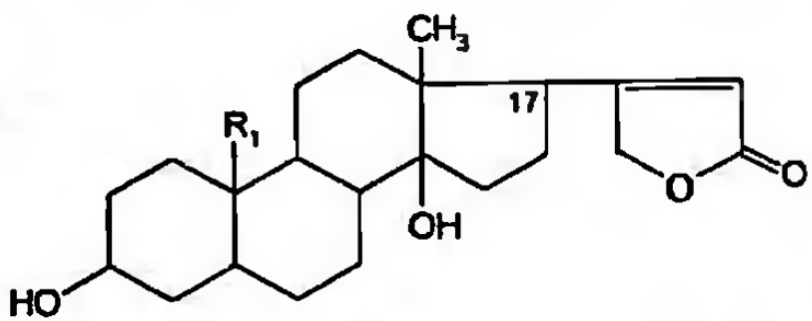
The size and degree of unsaturation varies with the source of the glycoside. Normally plant sources provide a fivemembered unsaturated lactone while animal sources give a six-membered unsaturated lactone.



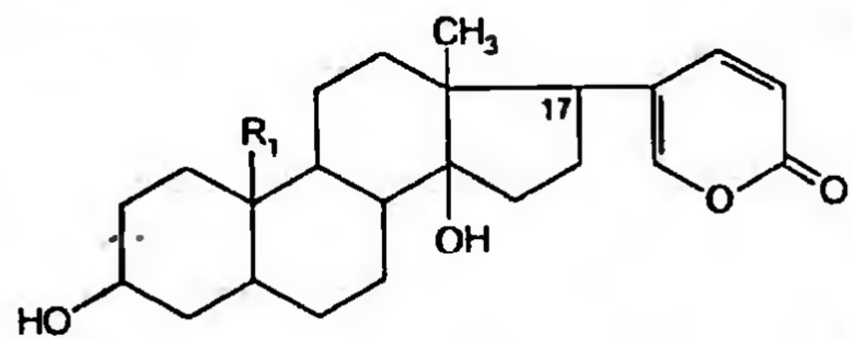
One to four sugars are found to be present in most cardiac glycosides attached to the 3β-OH group. The sugars most commonly used include L-rhamnose, D-glucose, D-digitoxose, D-digitalose, D-digginose, D-sarmentose, L-vallarose and D-fructose. These sugars predominantly exist in the cardiac glycosides in the β-conformation. The resence of acetyl group on the sugar affects the lipophilic character and the kinetics of the entire glycoside.



Two classes have been observed in nature—the cardenolides and the bufadienolides. The cardenolides have an unsaturated butyrolactone ring while the bufadienolides have a pyrone ring. The lactone of cardenolides has a single double bond and is attached at the C-17 position of steroidal nucleus. They are five-membered lactone ring and form a C23 steroids (Leguminosae, Cruciferae, Euphorbiaceae, etc.), while the lactone of bufadienolids have two double bond which is attached at the 17 α-position of the steroidal nucleus. They are six-memberd lactone ring and form C24 steroids (Liliaceae, Ranunculaceae).



Cardenolide



Bufadienolide

Distribution in nature

In plants cardiac glycosides appear to be confined to the Angiosperms. Cardenolides (see below) are the more common and are particularly abundant in the Apocynaceae and Asclepiadaceae, but are also found in some Liliaceae (e.g. *Convallaria*), and in the Ranunculaceae, Moraceae, Cruciferae, Sterculiaceae, Euphorbiaceae, Tiliaceae, Celastraceae, Leguminosae and Scrophulariaceae. The bufanolides occur in some Liliaceae (e.g. *Urginea*) and in some Ranunculaceae (e.g. *Helleborus*). In toad venoms the genins are partly free and partly conjugated with suberylarginine.

Some of the main genera containing cardiac glycosides are as follows:

Apocynaceae: *Adenium*, *Acokanthera*, *Strophanthus*, *Apocynum*, *Cerbera*, *Tanghinia*, *Thevetia*, *Nerium*, *Carissa* and *Urechites*;

Asclepiadaceae: *Gomphocarpus*, *Calotropis*, *Pachycarpus*, *Asclepias*, *Xysmalobium*, *Cryptostegia*, *Menabea* and *Periploca*;

Liliaceae: *Urginea*, *Bowiea*, *Convallaria*, *Ornithogalum* and *Rohdea*;

Ranunculaceae: *Adonis* and *Helleborus*; Moraceae: *Antiaris*, *Antiaropsis*, *Naucleopsis*, *Maquira* and *Castilla*;

Cruciferae: *Erysimum* and *Cheiranthus*;

Sterculiaceae: *Mansonia*; Tiliaceae; seeds of *Corchorus* spp.;

Celastraceae: *Euonymus* and *Lophopetalum*;

Leguminosae: *Coronilla*; and Scrophulariaceae.

In the latter family cardiac glycosides have been found only in the genus *Digitalis* if we include in this the plant which some botanists call *Digitalis canareniensis* and others place in the genus *Isoplexis*.

Physical and Chemical Properties of Cardiac Glycosides

Cardiac glycosides are bitter in taste, crystalline and odourless. With the exception of oubain, they are hydrophobic compounds slightly soluble in water but, freely soluble in alcohol. Treatment of cardiac glycosides with dilute acids results in hydrolysis of a glycosidic bond. More drastic conditions lead to elimination of C_{14} hydroxy group with the formation of 14,15-anhydro derivatives. In the presence of alkali, depending on the conditions, the following reactions could take place isomerization of the lactone ring from the unstable β-oriented to the stable α-orinted position and an addition reaction of the C_{14} hydroxy group on the lactone ring to give an isocardenolide.

Methods of Isolation and Chemical Tests of Cardiac Glycosides

It is relatively easy to obtain a crude extract of the plant material containing cardioactive glycosides by extracting the material with alcohol or alcohol-water mixture. However, isolating each of these glycosides in a pure crystalline form and without converting some of these glycosides into their hydrolytic products requires some of these cardioactive glycosides, especially the highly water soluble ones. Because of action of enzymes present in the plant materials, in which these glycosides occur, those glycosides, which contain terminal glucose units (e.g. lanatosides A, B, C, D and E and purpurea glycoside A and B), are liable to lose the glucose in the process of extraction. Inactivation of the enzymes by methods such as those referred to before or other methods is necessary to prevent the loss of such terminal glucose in the isolation of these glycosides. Enzymes capable of cleaving the linkages between two rare sugars or the linkages between rare sugar and aglycone are apparently absent in the plant materials, in which these glycosides occur, or they are not as active under the usual conditions of extraction.

The following procedures may be suggested for the isolation of a wide variety of steroidal cardioactive glycosides and the study of their aglycones and the 'rare' sugars they contain providing that the terminal glucose is not to be preserved intact, or when the glycosides in question do not contain such terminal glucose.

The fatty matter is removed from the plant material by extraction with petroleum ether. The defatted material is digested with water at 0–4 °C to remove the polysaccharides. The water extract is discarded and the marc is extracted with several portions of water — ethanol mixtures increasing the alcohol content progressively. The hydroalcoholic extract is concentrated to a smaller volume by distilling it in vacuum at 50 °C. Tannins are precipitated from the concentrate with lead hydroxide, and filtered. The filtrate is treated according to the rate of solubility of the glycosides in water as follows.

Chemical Tests

Cardiac glycosides give colour reactions with different reagents. These can be used for qualitative and quantitative purposes, as well as spray reagents on TLC.

Test for Sterols

Liebermann test for sterols: This test is characteristic of aglycones of the scillarenin type (the squill glycosides) and is due to the steroid part of the molecule. The test is carried by adding one drop of concentrated sulphuric acid to a solution of the glycoside in glacial acetic acid. A change in colour occurs from red, through violet and blue to green.

Test with antimony trichloride: Both the cardenolides and the bufadienolides (scilladienolides) give this colour reaction. When most of these cardioactive glycosides are heated with antimony trichloride and trichloracetic acid, a blue or violet colour is obtained.

Test for Aglycone Moiety

Legal test: This is a test for unsaturated lactones. The test may be carried out as follows: A small quantity (a few mg) of the glycoside (except scillaren) is dissolved in a few drops of pyridine. A drop of 2% sodium nitroprusside and a drop of 20% sodium hydroxide solution are then added. Production of a deep red colour gives a positive test.

Raymond test: A positive Raymond test depends on the presence of an activated methylene group (C_{31} in the lactone ring of the cardenolides). A small quantity of glycoside is dissolved in 1 ml of 50% ethanol, and this is followed by the addition of 0.1 ml of 1% solution of dinitrobenzene in ethanol (or methanol). To this solution two or three drops of 20% sodium hydroxide solution are then added. Appearance of a violet colour (which then changes to blue) gives a positive test.

Kedde reagent: This is widely used for spraying developed chromatograms of the cardenolides (or glycosides containing cardenolide aglycones). This reagent is essentially a modified form of the reagents used for the Raymond test. The Kedde reagent may be prepared by mixing equal volumes of 2% solution of 3,5-dinitrobenzoic acid in methanol and 5–7% aqueous solution of potassium hydroxide. The cardenolides react with this reagent to give a blue or violet colour, which fades in one or two hours.

Tollens test: A small quantity of glycoside is dissolved in a few drops of pyridine. Ammonia silver nitrate solution is then added. Liberation of silver gives a positive test.

Test for Sugars

Keller-Kiliani test: The 2-deoxy sugars (or the glycosides containing 2-deoxy sugars) respond to this test. This test may not be reliable if the sugar is acetylated. Glycoside is dissolved in glacial acetic acid containing a trace of ferric chloride; concentrated sulphuric acid containing the same amount of ferric chloride is placed at the bottom of the test tube with a pipette. An intense blue colour develops at the surface between the two reagents in 2–5 minutes spreading gradually into the acetic acid layer.

Xanthydrol test: This test is given by 2-deoxy sugars. When these are heated in the solution of xanthydrol in glacial acetic acid containing 1% HC1, a red colour is developed.

Quantitative Determination

The method adopted for determination of cardiac glycosides includes:

1. colourimetric method;
2. fluorimetric method;
3. gravimetric method;
4. biological method (determination of LD_{50});
5. immunoassay (radioimmunoassay);
6. RP-HPLC with UV or fluorometric detector.

Medicinal raw materials, containing cardiac glycosides

DIGITALIS LEAVES – DIGITALIS FOLIA

Digitalis (*Purple Foxglove Leaves*) consists of the dried leaves of *Digitalis purpurea* L. and *Digitalis grandiflora* L.

Family Scrophulariaceae.

The foxglove is a biennial or perennial herb which is very common in the UK and most of Europe, including some Mediterranean regions of Italy, and is naturalized in North America. It is produced commercially in Holland and Eastern Europe. In the first year the plant forms a rosette of leaves and in the second year an aerial stem about 1–1.5 m in height. The inflorescence is a raceme of bell-shaped flowers. The common wild form of the plant has a purple corolla about 4 cm long, the ventral side of which is whitish but bears deep purple eyespots on its inner surface. Many horticultural varieties exist, but these are of low therapeutic potency. The fruit is a bilocular capsule which contains numerous seeds attached to axile placentae.

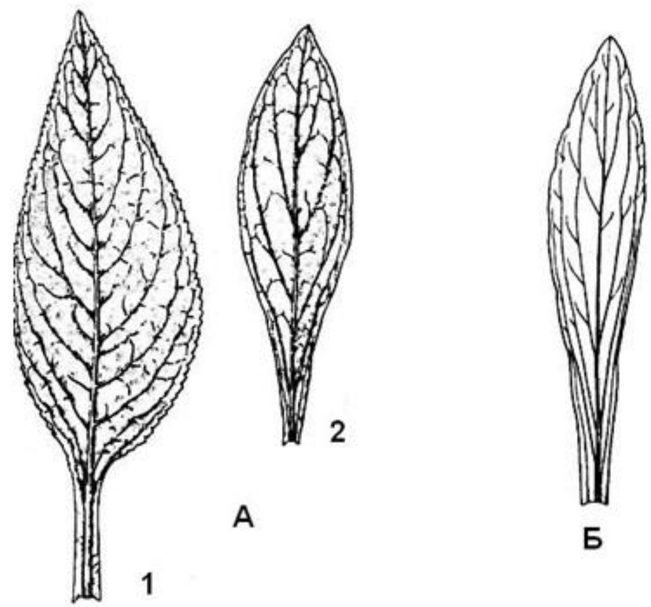
Digitalis grows readily from seed. In the wild state it is usually found in semi-shady positions. It grows well in sandy soil, provided that a certain amount of manganese is present, this element being apparently essential and is always to be found in the ash.

The inflorescence of Digitalis purpurea


The inflorescence of Digitalis grandiflora

Collection. Either first- or second-year leaves are permitted by the pharmacopoeias.
There has been a long-standing general belief that the pharmacological activity of leaves increases during the course of a day to reach a maximum in the early afternoon. Biological assays have given some support to this supposition and variations involving individual glycosides have also been reported.
After collection the leaves should be dried as rapidly as possible at a temperature of about 60°C and subsequently stored in airtight containers protected from light. Their moisture content should not be more than about 6%.

Macroscopical characters. Digitalis leaves are usually ovate-lanceolate to broadly ovate in shape, petiolate and about 10–30 cm long and 4–10 cm wide. The dried leaves are of a dark greyish-green colour. The lamina is decurrent at the base; apex subacute. The margin is crenate or dentate and most of the teeth show a large water pore.
Both surfaces are hairy, particularly the lower, and a fringe of fine hairs is found on the margin. The veins are depressed on the upper surface but very prominent on the lower. The main veins leave the midrib at an acute angle, afterwards branching and anastomosing repeatedly. The drug has no marked odour, but a distinctly bitter taste.



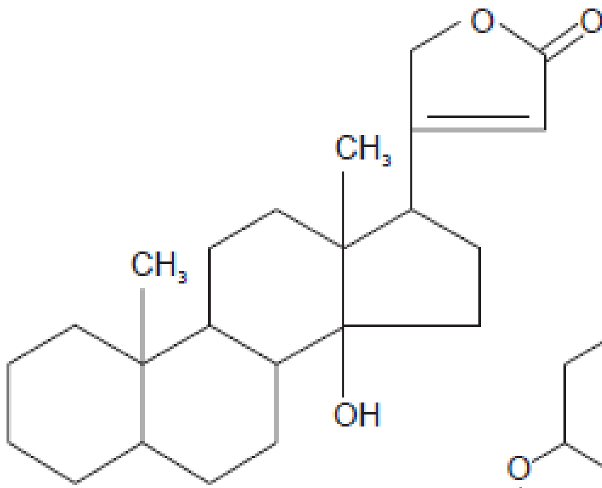
Leaves of foxgloves:A - Digitalis purpurea: 1 - root leaf; 2 - stem leaf; B – Digitalis lanata.

Chemical Constituents

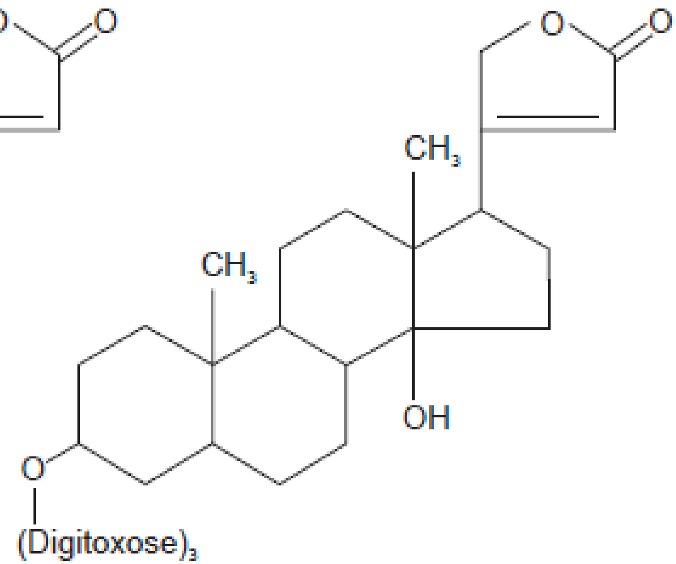
Digitalis leaves contains 0.2–0.45% of both primary and secondary glycosides. Purpurea glycosides A and B and glucogitoloxin are primary glycosides. Because of greater stability of secondary glycosides, and lesser absorption of primary glycosides a higher content of primary glycosides are not considered ideal and secondary glycosides are used.

Purpurea glycosides A and B are present in fresh leaves and by their hydrolysis digitoxin and glucose or gitoxin and glucose are obtained respectively. Hydrolysis of purpurea glycosides can take place by digipuridase (enzyme) present in the leaves. Digitoxin yields on hydrolysis digitoxigenin and three digitoxose. By hydrolysis of verodoxin, gitaloxigenin and digitalose are obtained. Digitalis leaves also contains glycosides like odoroside-H, gitaloxin, verodoxin and glucoverodoxin.

Verodoxin was found to potentiate the activity of digitoxin by synergism. Digitoxose and digitalose are desoxy sugars found only in cardiac glycosides and answers Keller–Killiani test. The important saponins include digitonin, tigonin and gitonin, and luteolin, a flavone responsible for the colour of the drug are also present in the leaves.



Digitoxigenin



Digitoxin

Uses

The foxglove is a widely used herbal medicine with a recognized stimulatory effect upon the heart. It is also used in allopathic medicine in the treatment of heart complaints. It has a profound tonic effect upon a diseased heart, enabling the heart to beat more slowly, powerfully and regularly without requiring more oxygen. At the same time it stimulates the flow of urine which lowers the volume of the blood and lessens the load on the heart. It has also been employed in the treatment of internal haemorrhage, in inflammatory diseases, in delirium tremens, in epilepsy, in acute mania and various other diseases. Digitalis has a cumulative effect in the body, so the dose has to be decided very carefully.

DIGITALIS LANATA LEAVES - DIGITALIS LANATA FOLIA

It consists of the dried leaves of *Digitalis lanata* J. F. Ehrh., belonging to family Scrophulariaceae.

Digitalis lanata is a biennial or perennial growing from a woody, horizontal rootstock. There is a tidy rosette before the spike goes up, and it is neatly arranged around the purple-tinged stems. The plant commonly forms a single, upright, more or less uniformly leafy stem that is partly ascending at the base. The plant send up these stems and flowers in usually in its second year. The stems are 0.3 to 0.6 meters in height, or about 13 to 26 inches.

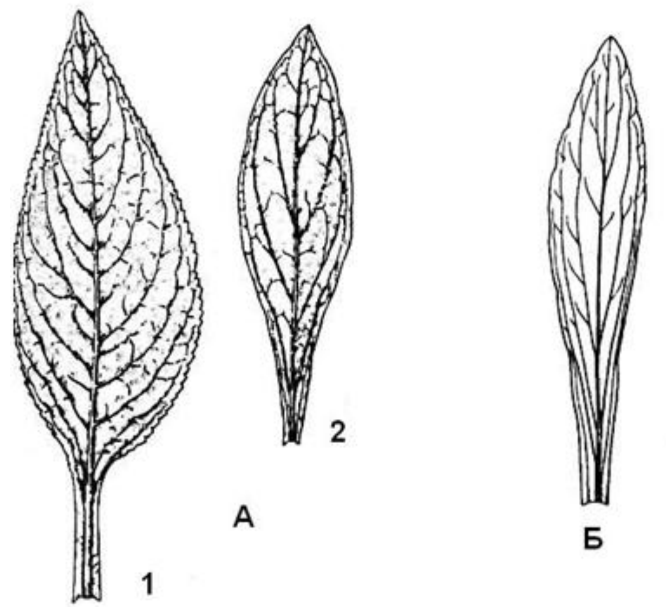
The leaves are moderately green in colour, woolly, veined, and covered with white hairs on the underside. They have a very bitter taste. The lower cauline leaves are 6 to 12 cm (sometimes to 20 cm) long and 1.5 to 3.5 cm wide, the upper cauline leaves are 4 to 10 cm long and lanceolate shaped, usually with entire margins, and with a distinctive mid-rib. The leaves along the stalks are alternate. The lower stem leaves wither by early flowering. Both flowers and stems are also woolly or hairy.

The inflorescence axis is densely covered with densely matted woolly hairs (tomentose), and the flowers are densely arranged into a raceme that is pyramidal in shape. The flowers are tubular and bell shaped, pale yellow to whitish with brown or violet lines (ferruginous reticulated markings), and the centre lobe of the lower lip is 8 to 13 mm long.

The fruit is a conical capsule with a blunt end topped by a short beak. The seeds which develop within are quadrangular or prismatic in shape, and are about 0.6mm broad and 1.1 to 1.3mm long.

Geographical Source
It is mainly found in Central and Southern Europe, England, California and India.

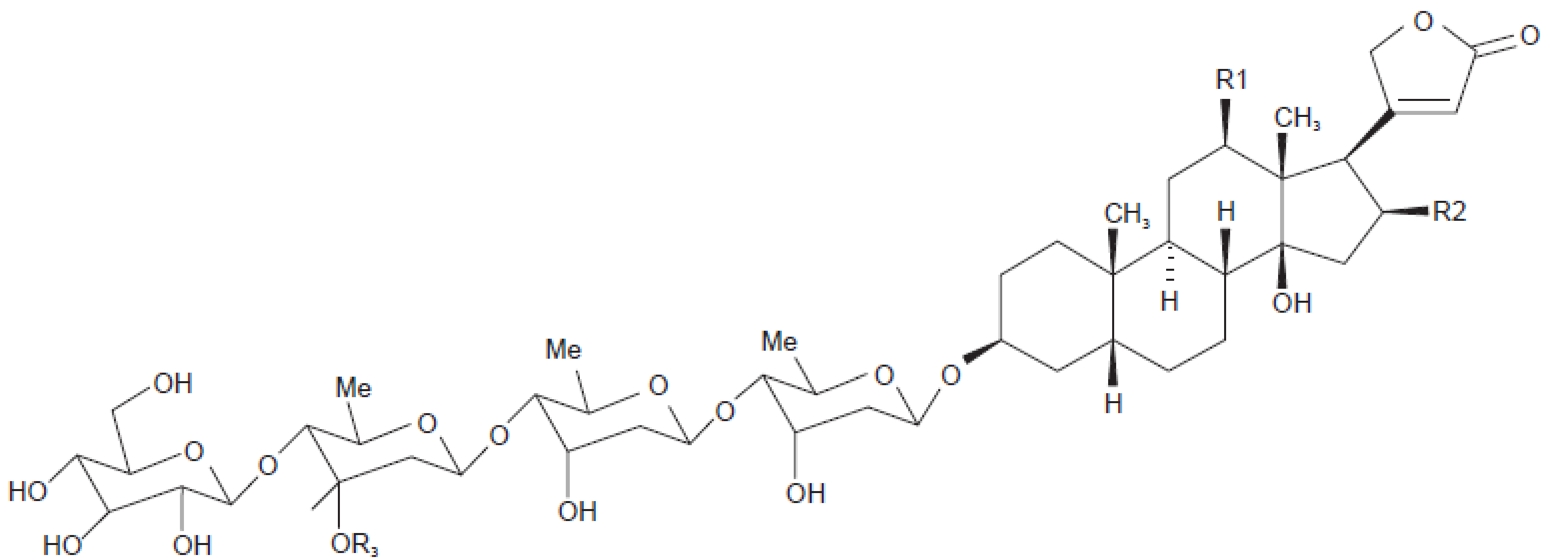
Characters. The leaves are sessile, linear-lanceolate to oblonglanceolate and up to about 30 cm long and 4 cm broad. The margin is entire, the apex is acuminate and the veins leave the midrib at a very acute angle. The distinctive microscopical characters are the beaded anticlinal walls of the epidermal cells, the 10–14-celled non-glandular trichomes which are confined almost exclusively to the margin of the leaf, and the glandular hairs found on both surfaces; some have bicellular heads and unicellular stalks, while others have unicellular heads and 3–10-celled, uniseriate stalks. As in *D. purpurea*, pericyclic fibres and calcium oxalate are absent.



Leaves of foxgloves:A - Digitalis purpurea: 1 - root leaf; 2 - stem leaf; B – Digitalis lanata.

Chemical Constituents

Digitalis lanata contains cardiac glycosides like lanatoside A, B, C and E. Lanatosides A and B are acetyl derivatives of purpurea glycosides A and B respectively. Hydrolysis of Lanatoside C yields digoxin, a crystalline active glycoside.



	R1	R2	R3
Lanatoside A	H	H	CH_3CO
Lanatoside B	H	OH	CH_3CO
Lanatoside C	OH	H	CH_3CO

Anthraquinone derivatives, similar to those found in *D. purpurea*, have been recorded in the leaves and a number of flavonoid glycosides characterized.

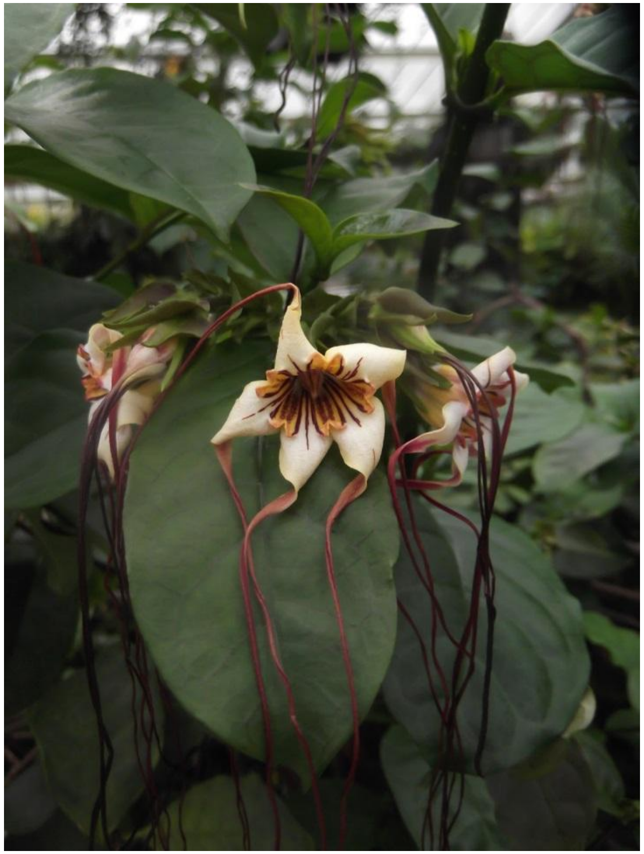
Uses

It has gained much importance in recent years because of the less cumulative effect and three to four times greater activity than *D. purpurea.* They have the same actions as that of the *D. purpurea.* It is the commercial source of digoxin. Employed in the treatment of auricular fibrillation and congestive heart failure. Their use should always be supervised by a qualified practitioner since in excess they cause nausea, vomiting, slow pulse, visual disturbance, anorexia and fainting.

STROPHANTHUS SEEDS (KOMBE SEEDS) - STROPHANTI SEMINA

Strophanthus consists of dried ripe seeds of *Strophanthus kombe* Oliv. deprived of their awns belonging to family Apocynaceae.

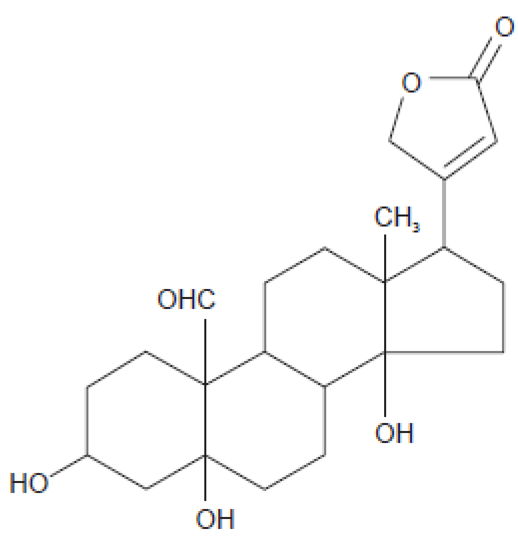
The plants are large, woody climbers, climbing on the large trees in the forests of Africa. Fruit consists of two divergent follicles which are dehiscent and many seeded. Each follicle is 30 cm long, 2.5 cm broad, tapering both at the apex and base. Mature and ripe fruits are collected in the month of June–July. After collection epicarp and fleshy mesocarp are removed and seeds separated from yellow-brown leathery endocarp and awns. Seeds are washed and dried. The seeds
are derived from anatropous ovules.



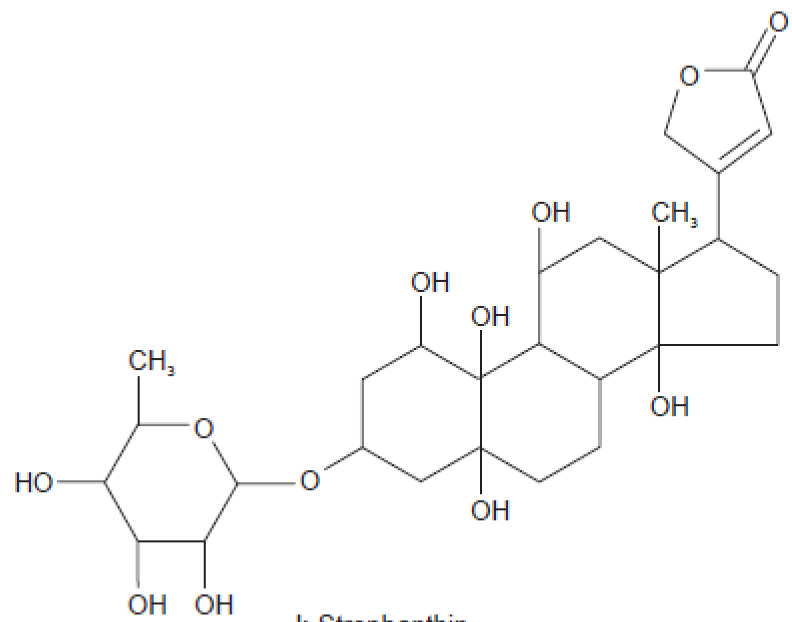

The official description of the seeds is lance-ovoid, flattened and obtusely edged; from 7 to 20 mm in length, about 4 mm in breadth and about 2 mm in thickness; externally of a light fawn colour with a distinct greenish tinge, silky lustrous form, a dense coating of flat-lying hairs (*S. Kombe*) bearing on one side a ridge running from about the centre to the summit; fracture short and omewhat soft, the fractured surface whitish and oily; odour heavy when the seeds are crushed and moistened; taste very bitter.

Chemical Constituents

The drug contains 8–10% cardiac glycosides known as k-strophanthin. k-strophanthin is a mixture of three glycosides, cymarin, k-strophanthin P and k-strophanthoside, which differ only through attached sugars and on hydrolysis yields same aglycone strophanthidin. It contains a sugar cymarose that is methoxy digitoxose which gives positive reaction for Keller–Killiani test. The drug also contains mucilage, resin, fixed oil, choline, trigonelline, and kombic acid—an acid saponin.



Strophanthidin



k-Strophanthin

Uses

The use of strophanthus in medicine is for its influence on the circulation, especially in cases of chronic heart weakness. As its action is same as that of digitalis, it is often useful

as an alternative or adjuvant to the drug. Believed to have greater diuretic power, it is esteemed of greater value in cases complicated with dropsies. Unlike digitalis it has no cumulative property.

CONVALLARIA HERB - CONVALLARIAE HERBA
CONVALLARIA LEAVES - CONVALLARIAE FOLIA
CONVALLARIA FLOWERS - CONVALLARIAE FLORES

Medicinal raw material consists of herb, leaves or flowers of *Convallaria majalis L*., *Convallaria keiskei Miq*, Convallaria transcaucasica Utkin ex Grossh. deprived of their awns belonging to family Liliaceae.

Convallaria majalis is an herbaceous perennial plant that often forms extensive colonies by spreading underground stems called rhizomes. The stems grow to 15–30 cmin tall, with one or two leaves 10–25 cm in long; flowering stems have two leaves and a raceme of five to fifteen flowers on the stem apex.
The flowers have six white tepals (rarely pink), fused at the base to form a bell shape, 5–10 mm in diameter, and sweetly scented; flowering is in late spring. The fruit is a small orange-red berry 5–7 mm in diameter that contains a few large whitish to brownish colored seeds that dry to a clear translucent round bead 1–3 mm in wide.

Convallaria majalis is a native of Europe, where it largely avoids the Mediterranean and Atlantic margins. An eastern variety, *C. majalis* var. *keiskei* occurs in Japan and parts of eastern Asia.

Chemical Constituents

The principal glycoside is convallatoxin which on hydrolysis gives strophanthidin and (−)-rhamnose. The plant contains many minor cardenolides, about 40 glycosides associated with nine different aglycones having been identified. Sugars not recorded elsewhere for cardiac glycosides are allose and the disaccharide rhamnosido-6-deoxyallose.
A number of flavonoid glycosides are also present in the leaves, and the roots contain a saponin, convallamaroside, which is a 22-hydroxyfuranostanol saponin with three independent sugar chains at C-1, C-3 and C-7.

Japanese lily of the valley, *Convallaria keiskei*, contains glycosides of convallagenin.

Uses

The lily of the valley, *Convallaria majalis* (Liliaceae) is much used on the continent of Europe and in herbal medicine for its cardioactive properties which are similar to those of digitalis but much less cumulative.

ADONIS HERB - ADONIS VERNALIS HERBA

Medicinal raw material consists of herb of *Adonis vernalis L.* deprived of their awns belonging to family Ranunculaceae.

A perennial herb, 10–40 cm high, with an erect stem. The leaves are sessile, finely divided, flowers solitary to 8 cm in diameter, of 10–20 elongated yellow petals. The fruit is an achene.

Pannonic area of Central Europe, Eastern Europe and West Siberia.

Chemical Constituents

Herb of *Adonis vernalis* contain more than 30 cardenolides, acting similarly to those of strophanthus; flavones including orientin, luteolin, and vitexin and cymarin is the major constituent..

Uses

It has been used in the Soviet Union to treat edema or swelling and as an alternative cardiac agent. In 1879, a Russian medical doctor, N. O. Buhnow, first introduced into medicine alcoholic extracts of the plant as a cardiac stimulant. In 1898, a mixture of the plant extracts with sodium bromide or codeine was suggested (by Vladimir Bekherev) to treat heart diseases, panic disorder, dystonia and mild forms of epilepsy. Aqueous infusions of the aerial parts of the plant have been traditionally used in Siberia against edema, cardiac edema and several other issues that are heart related, kidney diseases, and even malaria.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