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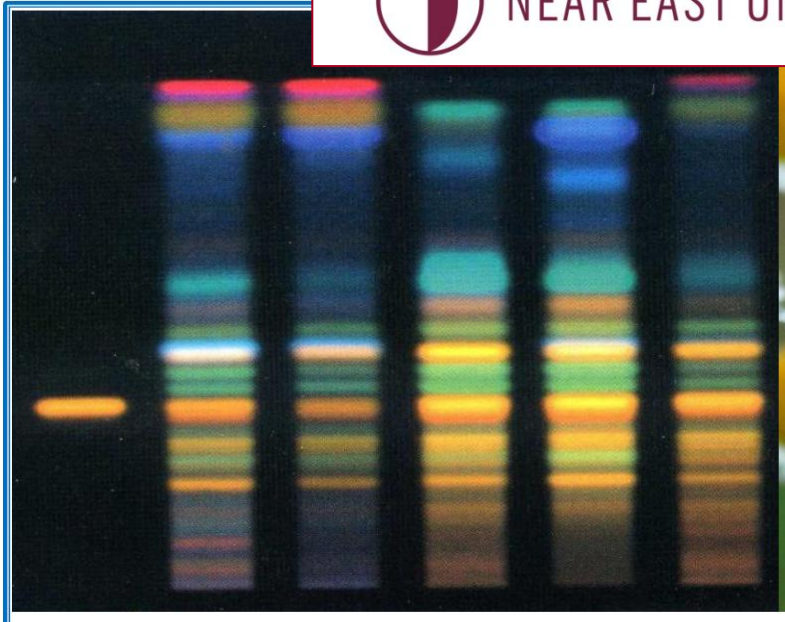

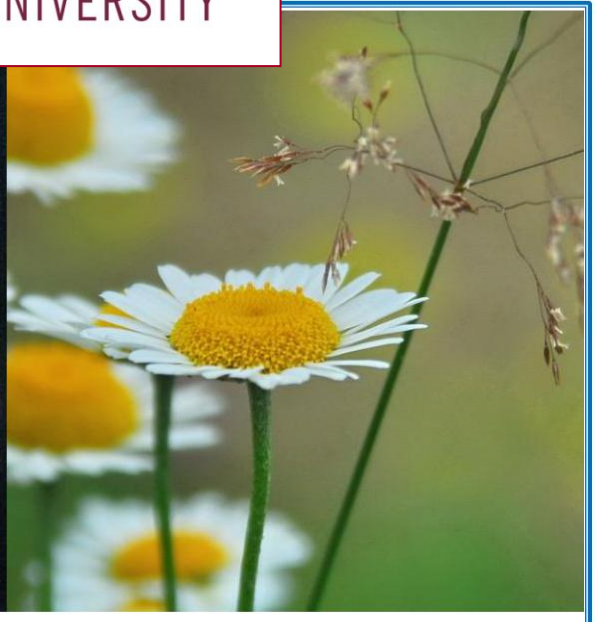

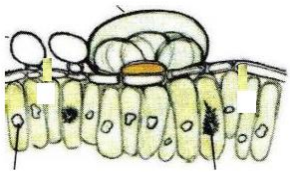

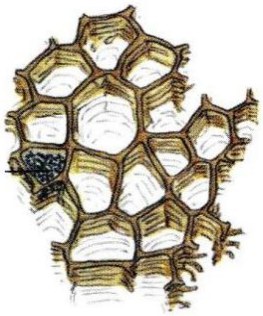

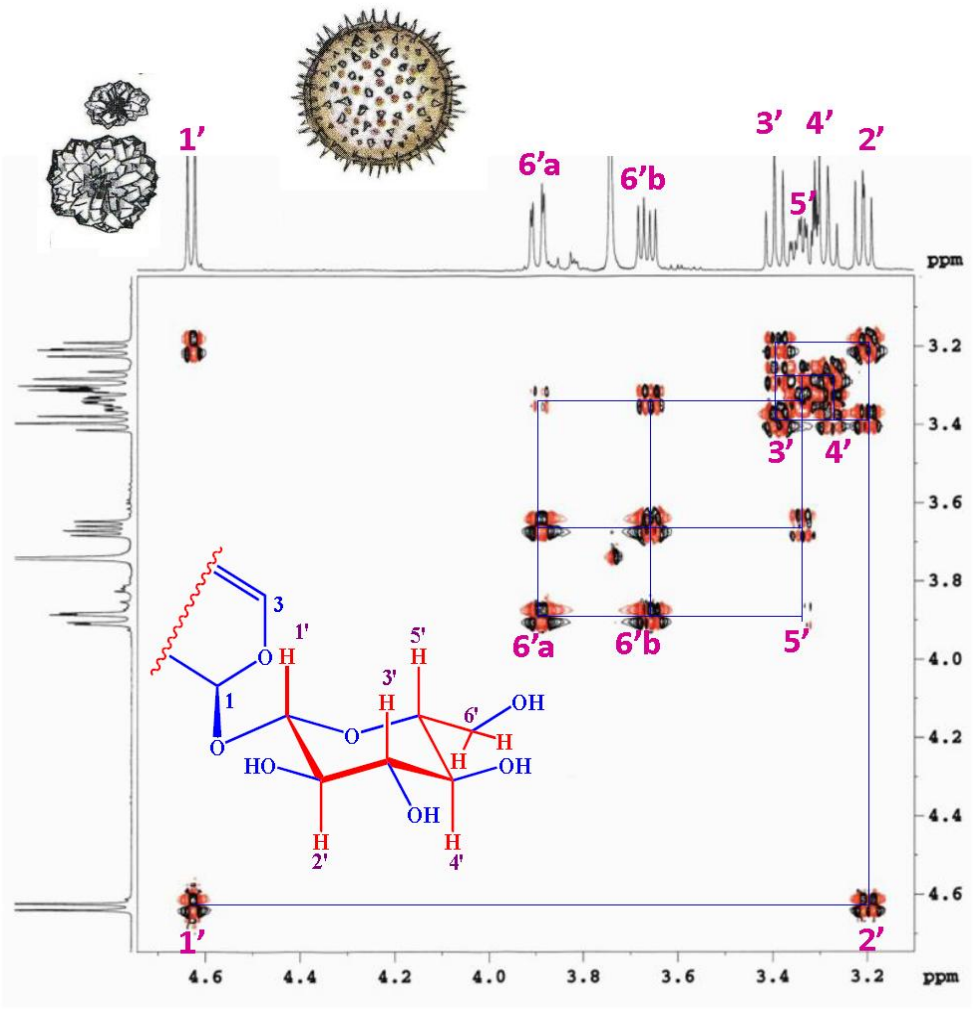


# NEPHAR 302 PHARMACOGNOSY I - Laboratory Manual

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**2015 – 2016 Fall Semester**

**Name Surname:**
**Number:**
**Microscope No:**
**Assistant:**

## Contents

# A. Introduction to Pharmacognosy

**Pharmacognosy** is the study of those natural substances, principally plants, that find use in medicine. Pharmacognosy is closely related to both botany and plant chemistry of which both have been originated from the earlier scientific studies on medicinal plants.

Pharmacognosy is "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources.

## Drugs

### a. Morphological Analysis

Morphological (=macroscopical) analysis is easy method for identifying crude drugs. Crude drugs are the dried, unprepared material of plant or animal origin. Most of the crude drugs are plant based. Plant form ranges from unicellular plants to the strongly differentiated higher plants. Characteristically the higher plants consist in the vegetative phase of roots, stems and leaves with flowers, fruits and seeds forming stages in the reproductive cycle. Some of the drugs are exudates (gums, resins) or they have been obtained by a secondary process (essential oils, fixed oils etc.). Due to the lack of modern analysis methods, the quality of crude drugs were based on the five senses (e.g., **colour, odour, taste, shape, size, dimensions, surface characters, fracture and texture**). With another words, morphological characters of crude drugs have been used for the quality assurance, authentication, and identification of adulteration.

*It is important to interpret morphological and anatomical descriptions of crude drugs as found in pharmacopoeias and allied works and also to record adequately the features of whole or powdered drugs and adulterants of commercial significance.*

**Nomenclature:**

*The pharmaceutical names generally consist of two words. One of these is related to the scientific name of the plant from which the drug derives while the second indicates the plant part (bark, leaf etc.) used. The following terms are used to indicate the parts of plants:*

**Radix = root**: The term does not completely coincide with the botanical concept. A drug termed a radix may sometimes also contain rhizomes.

**Rhizoma = rhizome**; A subterranean stem, generally carrying lateral roots.

**Tuber**: A nutritious subterranean organ, which, in a botanical sense, is a rhizome. A tuber is a thick organ, mainly consisting of parenchymatous storage tissue (generally containing starch) and a small proportion of lignified elements.

**Bulbus = onion**: Botanically, an onion is a stem, surrounded by thick nutritious leaves that are usually low in chlorophyll content.

**Lignum = wood**: Drugs for which this term is used are obtained from plants with secondary thickening and consist of the woody parts of the xylem.

**Cortex = bark**: Barks are obtained from plants with secondary thickening and, unlike the botanical definition of the term, they consist of all the tissues outside the cambium. Such drugs can be collected from roots, stems and branches.

**Folium = leaf**: Leaf consists of the middle leaves of the plant.

**Flos = flower**: The crude drug may consist of single flowers and/or entire inflorescences.

**Fructus = fruit**: The pharmacognostical term is not always synonymous with the botanical one. Thus, the drug drug Cynosbati fructus cum semen (rose hips) is, botanically speaking, a swollen receptacle carrying the true fruits (nuts). Also the second part of the term - semen - is thus not correct from a botanical standpoint as semen is the pharmaceutical term for seed (see below). There is also another crude drug consisting only of the receptacle without fruits. The pharmaceutical name for this crude drug is Cynosbati fructus sine semen.

**Pericarpium** = fruit peel or pericarp which is the common botanical term.

**Semen = seed**: The drug can consist either of the seed, as removed from the fruit, or of a part of the seed, as in Colae semen, which does not contain the testa or seed coat.

**Herba = herb**: The crude drug consists of the aerial parts of the plant; thus, stems as well as leaves, flowers and fruits, if any, are included.

**Aetherolum** = **essential or volatile oil** is, a product obtained from plant material. It usually possesses a distinctive odour and consists of a complex mixture of comparatively volatile components.

**Oleum = oil**, is a fixed oil prepared from plant material by pressing.

**Pyroleum = tar**, is prepared by dry distillation of plant material.

**Resina = resin**, is obtained either from secretory structures in certain plants or by distillation of a balsam (see below). In the latter case, it is the residue after distillation.

**Balsamum = balsam**, is a solution of resin in a volatile oil and is generally produced by special cells in the plant.

## Quality control of crude drugs

Quality specifications for crude drugs are given in pharmacopoeias and handbooks. The specifications are usually presented as a *monograph* of the crude drug. A monograph usually comprises the following items:

1. *The name and origin of the crude drug.*
2. *Characters.*
3. *Identity, comprising macro- and microscopic morphological characters and chemical tests.*
4. *Purity tests.*
5. *Quantitative determinations.*
6. *Instructions for storage.* Preparations of crude drugs

The most important use of crude drugs today is for extraction of pure, pharmacologically active compounds to be incorporated into tablets and other ready-made drugs. There is, however, still a market for simple preparations of crude drugs like teas and extracts which are sold as herbal remedies, mostly for self-medication.

**Grinding of crude drugs**
**Herbal "Teas"**
**Extract (dry-, soft-, fluid extracts, tinctures)**

## Herbal "Teas"

These preparations consist of coarse powders (particle size 2-4 mm). A herbal tea may contain only one crude drug but it may also be a mixture. The consumer usually prepares his remedy as an ***infusion,*** i.e. by pouring boiling water over the plant material, stirring and allowing the mixture to steep for a short time, whereupon the plant parts are removed by decantation or filtration and the aqueous extract drunk while it is still warm.

On an average a heaped teaspoonful of a crude drug weighs about 2.5 g but there are considerable differences depending on which plant parts are used. Thus this volume of chamomile flowers weighs only 1 g, of a leaf drug 1.5 g and of a root or a bark about 4.5 g. The amount of water to be taken is usually stated as a cup which means a volume of 150-250 ml.

Herbal teas are sometimes prepared as ***decoctions*** which means that the plant parts are boiled with the prescribed quantity of water. In some cases, in particular for crude drugs containing mucilage, an extract is prepared with cold water.

As water is the solvent for preparation of remedies from herbal teas one might expect that these remedies contain only very polar substances. However, investigations, aiming at isolation of pharmacologically active compounds from the preparations, have shown that an aqueous extract contains compounds which, when obtained in a pure state, turn out to be almost insoluble in water. The reason for this is that an aqueous extract of a plant material is very complicated and contains compounds which act as solubilizers for less polar compounds.

## Extracts

Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent used in making the extract.

In ***dry*** **extracts** *(**ex tract a sicca**)* all solvent has been removed. ***Soft*** **extracts** (***extracta spissa***) and ***fluid*** **extracts** *(**extracta fluida**)* are prepared with mixtures of water and ethanol as solvent.

A soft extract contains 15-25 *%* residual water. A fluid extract is concentrated to such an extent that the soluble constituents of one part of the crude drug are contained in one or two parts of the extract. *Tinctures* are prepared by extraction of the crude drug with five to

ten parts of ethanol of varying concentration, without concentration of the final product. For both extracts and tinctures the weight-ratio drug/extract should always be stated.

Thus if 100 g of a crude drug yields 20 g of dry extract the ratio is 5:1. Consequently, if the same amount of crude drug is used to prepare 1000 g of tincture, the ratio is 1:10. By definition the crude drug/extract ratio for a fluid extract is 1:1 or 1:2.

***Choice of solvent***: The ideal solvent for a certain pharmacologically active constituent should:

1. Be highly selective for the compound to be extracted.
2. Have a high capacity for extraction in terms of coefficient of saturation of the compound in the medium.
3. Not react with the extracted compound or with other compounds in the plant material.
4. Have a low price.
5. Be harmless to man and to the environment.
6. Be completely volatile

## Extraction procedures

**Maceration, Percolation, Countercurrent extraction**

The ethanol is usually mixed with water to induce swelling of the plant particles and to increase the porosity of the cell walls which facilitates the diffusion of extracted substances from inside the cells to the surrounding solvent. For extraction of barks, roots, woody parts and seeds the ideal alcohol/water ratio is about 7:3 or 8:2. For leaves or aerial green parts the ratio 1:1 is usually preferred in order to avoid extraction of chlorophyll.

**Maceration**: This is the simplest procedure for obtaining an extract and is suitable both for small quantities of drug and for industrial production. ***Simple maceration*** is performed at room temperature by mixing the ground drug with the solvent (drug/solvent ratio: 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring. The extract is then separated from the plant particles by straining. The procedure is repeated once or twice with fresh solvent. Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.

**Percolation***: Simple percolation* is a procedure in which the plant material is packed in a tube-like percolator which is fitted with a filter sieve at the bottom. Fresh solvent is fed from the top until the extract recovered at the bottom of the tube does not contain any solute. This is a slow and costly process requiring large quantities of fresh solvent.

A technical problem in percolation is to ensure an equal How of solvent through the mass of crude drug powder. The drug should not be too finely ground to allow a reasonably fast passage of the solvent. A particle size of 1-3 mm is usually sufficient. Before the material is loaded into the percolator it should be moistened with the solvent and allowed to swell. It is then carefully packed into the percolator in such a way that the layer formed is as uniform as possible. Solvent is administered at the top and passes through the drug. The extract is collected at the bottom or is passed on to the next percolator if a battery is used. Transport of solvent can be achieved by gravity or by pumping.

**Countercurrent extraction**: This is a continous process in which the plant material moves against the solvent. Several types of extractors are available. In the ***screw extractor*** the plant material is transported by a screw through a tube and meets the solvent which is pumped in the opposite direction.

**Extraction with supercritical fluids**: At a sufficiently low temperature a gas may be made to liquefy by applying pressure to reduce the volume. However, there is a temperature above which it is impossible to liquefy the gas no matter how great a pressure is applied. This temperature is called the ***critical temperature***. The minimum pressure necessary to bring about liquefaction at the critical temperature is called the ***critical pressure.*** The combination of critical pressure and critical temperature is characteristic of the particular substance and is called the *critical point.* Gases at temperatures and pressures above the critical point are called *supercritical gases* or *supercritical fluids.* Only gases which can be converted into the supercritical state at attainable pressures and temperatures can be considered for extraction use.

| ***Critical temperatures and pressures*** | | |
|---|---|---|
| **Fluid** | Critical temp., °C | Critical pressure, bar |
| Ethylene | 9.3 | 50.4 |
| Carbon dioxide | 31.1 | 73.8 |
| Ethane | 32.3 | 48.8 |
| Nitrous oxide | 36.5 | 72.7 |
| Propylene | 91.9 | 46.2 |
| Propane | 96.7 | 42.5 |
| Ammonia | 132.5 | 112.8 |
| Hexane | 234.2 | 30.3 |
| Water | 374.2 | 220.5 |

***Purification and concentration of extracts***:
The methods used are ***decantation, centrifugation*** *and* ***filtration***.

For the manufacture of fluid and soft extracts the clarified extract must be concentrated. Preparation of a dry extract requires complete removal of the solvent. Concentration is a tricky stage in the process in which many chemically labile compounds may undergo degradation, mainly due to the temperature. Concentration *in vacuo* is therefore the preferred method by which the extract can be kept at 25-30°C during the whole procedure. Several types of concentrators are available.

***Drying of extracts***:

**The concentrators** (rotary evaporator) can be used for production of fluid and soft extracts but are not suitable for complete drying of an extract.

Drying in **cabinet driers**: Hot air (60 – 80 °C) is blown over the shelves.

Drying in **atomizers** (spray drying) is suitable for industrial production.

**Freeze-drying**: Freeze-drying (lyophilization) is a very mild method. Frozen material is placed in an evacuated apparatus which has a cold surface maintained at -60 to -80 °C. Water vapour from the frozen material then passes rapidly to the cold surface.

## b. Microscopical Analysis

Aim of the microscopic analysis of the powdered crude drugs is also identification and authentication. The structure of cell wall, cell shape and cell contents are microscopical characters of the medicinal plants and they are of value in identification and in the detection of adulteration.

The aim of the microscopical examination of crude drugs:

i. The determination of the size, shape and relative positions of the different cell and tissues
ii. The determination of the chemical nature of the cell wall
iii. The determination of the form and chemical nature of the cell contents.

This method is useful for identifying herbal drug powders and for distinguishing species with similar morphological characters. By means of microscopic techniques, structural and cellular features of herbs are examined in order to determine their botanical origins and assess their qualities. These are:

**THE CELL WALL**

Cellulose walls, lignified walls, suberized and cutinized walls, musilaginous cell walls, chitinous cell walls

PARANCHYMATOUS TISSUE

**THE EPIDERMIS**

**EPIDERMAL TRICHOMES**

THE ENDODERMS

CORK TISSUE

COLLENCHYMA

**SCLEREIDS** (Sclereid or Stone cells)

XYLEM (Tracheids, vessels or trachea, xylem fibres, xylem paranchyma)

PHLOEM (Sieve tubes, companian cells, phloem paranchyma and secretory cells)

SECRETORY TISSUES (Secretory cells, secretory cavities, canals and latex tissue)

**ERGASTİC CELL CONTENTS**

Starch, proteins, fixed oil, gums, musilages, volatile oils, crystals

**Reagents in Microscopical Studies:**

**Water, distilled**: A useful mountant for starches. Sections which have been bleached with solution of chlorinated soda or similar reagent may be freed from the bubbles of gas which they frequently contain by placing them in freshly boiled distilled water.

**Chloral hydrate solution:** (chloral 50 g, water 50 ml): A valuable and widely used clearing agent. Dissolved starch, have an effect when heating the preparate.

**Sartur Reagent** (**Sar**ım ÇELEBİOĞLU & **Tur**han BAYTOP):

**Composition**:

| | |
|---|---|
| Lactic Acid | 60 mL |
| Lactic Acid saturated with sudan III (at cold) | 45 mL |
| Aniline | 2 g |
| Iode | 0.2 g |
| Potasium iodide | 1 g |
| Alcohol 95% | 10 mL |
| Distilled Water | 80 mL |

***Lactic Acid***: Clarify sections and preparates

***Sudan III***: Stains oils and suberized walls (cork tissues) to orange-brown. It is also usefulin the examination of secretory cells and ducts.

***Aniline:*** Reacts with lignin in acidic conditions and give yellow colour (stains the schlerenchyma tissues, xylem, stone cells and scleroids)

**Iode**: Reacts with starch and stains to blue-purple.

**Potasium iodide**: It is essential to solve iode.

**Alcohol** 95% and **water** are the supporting elements for the preparation of reagent.

**<u>Some other reagents used in microscopy</u>:**

**Ethanol**. Different strengths are used for preserving material and for hardening. Alcohol acts as a clearing agent by dissolving oils, resins, chlorophyll, etc. It does not dissolve gums and mucilages (therefore a useful mountant for drugs containing them).

**Chloral hydrate with iodine.** When used cold, causes shrunken cells and starch grains to expand. The iodine stains starch or hemicelluloses.

**Clove oil.** A useful clearing agent for powders containing much oil.

**Chlor-zinc-iodine solution (syn. Schulze's solution).** Prepared by adding a solution of zinc chloride (zinc chloride 20 g; water 8.5 ml) dropwise to a solution of potassium iodide (1.0 g) and iodine (0.5 g) in water (20 ml) until a precipitate of iodine forms which does not disappear on cooling. This requires about 1.5 ml. Used as test for walls containing celluloses. Iodine solution followed by sulphuric acid gives similar results.

**Ether-ethanol.** A defatting agent.

**Glycerin, dilute.** One volume of glycerin is mixed with two volumes of distilled water. A useful mountant for preparations which may be left for some time, as it does not dry up. It has some clearing action, but is much inferior in this respect to chloral hydrate. It is not a good mountant for starch, as the grains tend to become transparent and striations, etc., are difficult to see; water is preferable.

**Iodine water, *BP*.** This gives a blue colour with starch and hemicelluloses.

**Mercury-nitric acid solution *BP* (syn. Millon's Reagent).** Test for protein-containing materials e.g. aleurone grains, wool and silk.

**Phloroglucinol solution.** A 1% solution in 90% ethanol with hydrochloric acid as a test for lignin.

**Picric acid solution.** A saturated solution in water which is used to stain aleurone grains and animal fibres.

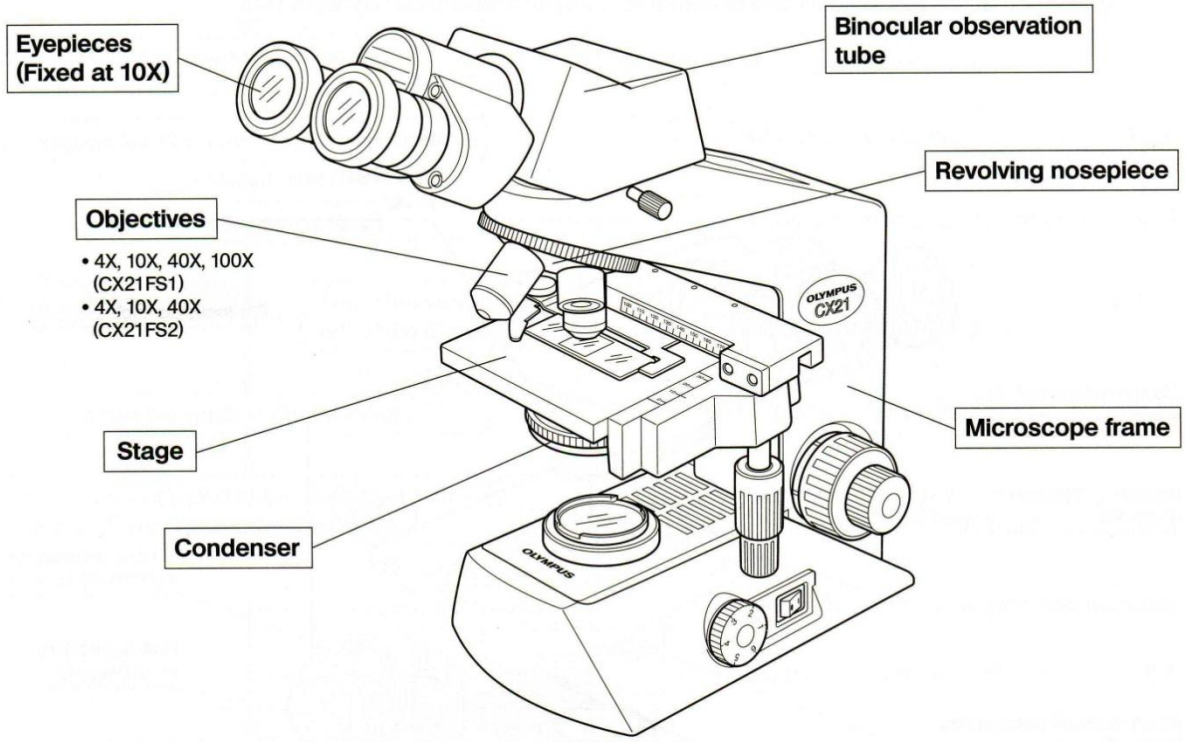
**Ruthenium red, solution of, *BP*.** Stains many gums and mucilages. It must be freshly prepared.

**Sodium hypochlorite solution.** The *BP* includes a strong and weak solution; for use see 'Clearing, Defatting and Bleaching'.

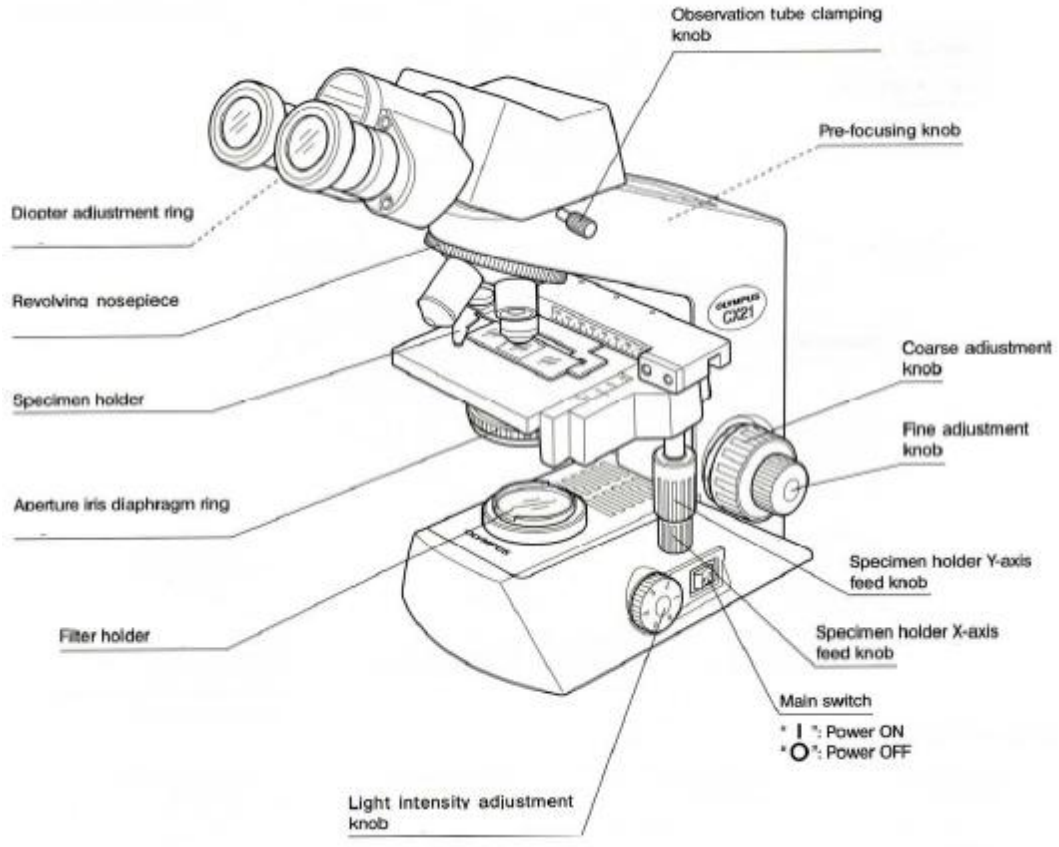
**Sulphuric acid 80%.** Concentrated sulphuric acid causes rapid charring, but dilutions containing 80% or less form useful reagents. The behaviour of cotton, wool, chalks, calcium oxalate and sections of strophanthus seeds should be noted. The acid dissolves cellulose and lignified walls, but has little action on suberin.

**MICROSCOPE** (OLYMPUS CX21)

**A. The Main Parts of a Microscope:**




**B. Functional Parts**



**GENERAL RULES IN MICROSCOPICAL STUDIES (Important):**

1. Before examining your samples you should clean your microscobe, arrange the mirrors.
2. Examine the organoleptic specifications of the sample and write down for your report.
3. Prepare your samples carefully as described.
4. Prepare your samples with Sartur R. first and write down the colourings of the sample.
5. Prepare your samples with cloralhydrate and draw the cells, tissues etc. for your report
6. Write down the scales you use.
7. After drawing the element you see, you need to get approval for your drawings.
8. When you work with unknown samples you should note the code on your sample and need to use your lab guidebook.
9. You should write your analysis as a report to your lab book.
10. When you work with powdered samples you need to keep your cachets closed and keep clean the needles you use all the time. You should make sure that the reactive bottles are closed properly after you use.
11. You need to clean your microscope and bench before you leave the laboratory.

**What you should bring during the Pharmacognosy Laboratory I**

1.White coat

2.Cleaning tissues

3. Matches

4. Notebook (A4)

## c. Chemical Analysis (PHYTOCHEMISTRY)

### i. EXTRACTION OF PLANT MATERIAL

### ii. IDENTIFICATION*, SEPARATION AND ISOLATION OF CONSTITUENTS

Sublimation
Distillation
Fractional Liberation
Fractional Crystallization

**CHROMATOGRAPHICAL STUDIES**

The aim of chromatographical studies is qualitative and/or quantitative detection, analysis and preparative isolation of plant primary and mainly secondary metabolites.

ADSORPTION CHROMATOGRAPHY
THIN LAYER CHROMATOGRAPHY (TLC)*
- High-Performance TLC

PARTITION CHROMATOGRAPHY
- Counter-current extraction (CCC)
- High-Speed CCC
- Droplet CCC

LIQUID CHROMATOGRAPHY
- Low-Pressure LC
- Medium-Pressure LC
- High-Performance LC (HPLC) – High-Speed LC
- Ultra-HPLC

GAS-LIQUID CHROMATOGRAPHY

CAPILLARY-COLUMN GAS CHROMATOGRAPHY
GEL FILTRATION (MOLECULAR SIEVE)
ELECTROCHROMATOGRAPHY
AFFINITY CHROMATOGRAPHY

### iii. CHARACTERIZATION OF ISOLATED COMPOUNDS

UV, IR
NMR: 1D-NMR ($^{1}$H-NMR, $^{13}$C-NMR)
2D-NMR
- **COSY** : Proton COrrelation SpectroscopY
- **TOCSY**: Total COrrelation SpectroscopY
- **HOHAHA**: HOmonuclear HArtmann HAhn
- **HMQC**: Heteronuclear Multiple Quantum Correlation
- **HSQC**: Heteronuclear Single Quantum Coherence
- **NOESY**: Nuclear Overhauser Enhancement SpectrescopY
- **ROESY**: Rotating-frame Overhauser SpectrescopY
- **HMBC**: Heteronuclear Multiple Bond Correlation

Mass Spectrometry
- *Ionization Methods*: **EI-, ESI-, FAB-, FD-Mass etc.**

X-Ray Crystallographic
Optical Rotation
- Optical Rotatory Dispersion (ORD

## d. ASSAY for Biological and Pharmacological Activity

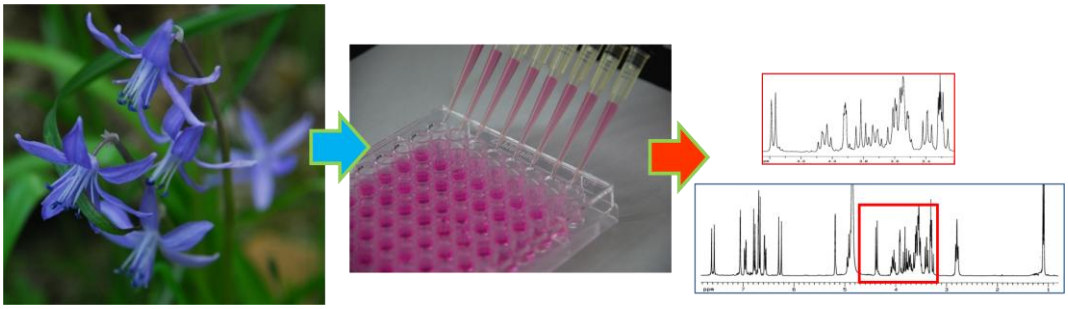
I. **Activity-Guided Studies (= Bioassay-guided isolation)**: The aim is to isolate active compounds from the plant extracts. This process is hampered by the lack of knowledge of the chemical properties of the compounds. The isolation process must therefore be monitored by testing all isolated fractions for the activity. Only the fractions which give a positive result in the test are subjected to further separation steps and eventually the pure active compound(s) is (are) obtained. This procedure is termed ***bioassay- guided isolation***. Example: *Anti-inflammatory compounds* (cyclooxygenase, COX inhibiton)

> **Warning**: *It is important to get some preliminary information about polarity, charge, molecular size and stability of the active compounds in order to design a rational isolation procedure.*

II. **Chemical Structure Oriented Isolation Studies**: A certain type of chemical classes having potential activity are targeted in the isolation. Example: *Alkaloids, saponins, phenolics or iridoids*

High-performance liquid chromatography (HPLC) is a very powerful and versatile chromatographic technique for the separation of natural products in complex matrices, such as crude extracts for selective detection. The method is widespread and has been adapted to the analysis of a broad range of NPs generally without the need for complex sample preparation. The choice of the detection method in HPLC is crucial because of the diversity of natural products. HPLC can be combined with UV (ultraviolet), DAD (photodiode array detection), FD (fluorescence detection), MS (mass spectrometry), MS-MS, and NMR (nuclear magnetic resonance) which are called hyphenated techniques. GC-MS (Gas Chrom.-Mass Spectrometry) is another hyphenated technique suitable for ananlysis of volatile compounds like terpenoid constituents of essential oils.

III. **High-Throughput Screenings (HTS):**



This is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. Using robotics, data processing and control software, liquid handling devices, and sensitive detectors, High-Throughput Screening allows a researcher to quickly conduct millions of chemical, genetic or pharmacological tests.

HTS is used by the pharmaceutical industry for detection of leads to new drugs. This technique can be applied to detect pharmacological activities of extracts, fermentation broths or cell cultures. It can also be used to monitor the isolation of active compounds from such material. HTS is defined as *the process by which large numbers of compounds can be tested, in an automated fashion, for activity as inhibitors (antagonists) or activators (agonists) of a particular biological target, such as a cell- surface receptor or a metabolic enzyme.*

## Books for further reading.

**Pharmacognosy, Phytochemistry, Medicinal Plants**
J. Bruneton, Lavoisier Publishing, London New York, 1999

**Fundamentals of Pharmacognosy and Phytotherapy**
M. Heinrich, J. Barns, S. Gibbons, E.M. Williamson, Churchill Livingstone, London 2004

**Trease and Evans' Pharmacognosy**
W. Charles Evans BPharm BSc PhD DSc FIBiol FLS FRPharmS, WB Saunders Company Ltd., London, 2009

**Pharmakognosie, Phytopharmazie**
R. Hänsel & O. Sticher, Springer Verlag, Würzburg, 2007

**Bitkisel Drogların Anatomik Yapısı**
Asuman Baytop, İstanbul Üniversitesi Eczacılık Fakültesi Yayınları, İstanbul, 1987

**Pulver – Atlas der Drogen – der deutschsprachigen Arzneibücher**
Walter Eschrich, Deutscher Apotheker Verlag, Stuttgart, 2009

**Pharmazeutische Biologie.4.** Drogenanalyse II: Inhaltsstoffe und Isolierungen
E. Stahl, W. Schild, Gustav Fischer Verlag, Stuttgart, 19881

**Drogen-analyse.** Dünnschichtchromatographische Analyse Von Arzneidrogen
H. Wagner, S. Bladt, E.M. Zgainski, Springer Verlag, Berlin, 1983.

**Drugs of Natural Origin – A Textbook of Pharmacognosy**
Gunnar Samuelsson, Apotekarsocieteten, Sweden, 2004.

**Classics in Spectroscopy – Isolation and Structure Elucidation of Natural Products**
Stefan Berger, Dieter Sicker, Wiley – VCH, Weinheim, 2009

**Phytochemical Methods**
J. B. Harborne, Chapman and Hall, London, 1973

## Laboratory Studies in Pharmacognosy

### a. Morphological Analysis

A drug collection consists of Kampo Medicine of Japan is presented in the show room at the second flor of the building. **Each student will prepare a short article for a drug selected from this collection**.

### b. Microscopical Analysis

Microscopical Studies will be performed to learn ergastic compounds, epidermal trichomes, stomata and glandular tissues, some basic cell- and tissue-types and flores elements of the powdered drugs. The systematic approach to identification of powdered drugs can proceed in a number of ways. In microscopical studies all methods depend on the microscopical recognition of characteristic cell types and cell contents. Identification can be made by reference drawings, tables and illutrations.
In the first five weeks of laboratory studies, the significant selected examples will be studied.

### c. Phytochemical Analysis (PHYTOCHEMISTRY)

In the second part of the laboratory studies, extraction and chromatographical (Thin Layer Chromatography = TLC) methods will be used. The aim of these type of studies is to reach new compounds which will be used as a led in drug development studies. These kind of studies need a long-term laboratory studies and are the subject of the pharmacognostical researches. With the application of the modern emerging techniques in the field of pharmacognosy is likely to change the face of pharmaceutical research, drug development and discovery

### d. Assay for Biological and Pharmacological Activity

In drug discovery studies, simplest methods are used to select candidates either in synthetic chemistry laboratories or natural products chemistry. A biological system is generally used to report on the potency of the product. The system may be animal, organ, tissue, or cell culture based. Some of the assay is based on the enzyme inhibition.

Some simple examples used in activity studies:

**Brine Shrimp Lethality:** A Rapid General Bioassay for Bioactive Compounds

**Crown Gall Tumors on Potato Discs:** An Animal-Sparing Bioassay for Antitumor Compounds,

**Frond Inhibition of *Lemna* (duckweed):** A Bioassay for Plant Growth Stimulants and Inhibitors

**Antibacterial, antiviral and antifungal activities** agains selected microorganisms

**Yellow Fever Mosquito (YFM) Test:** A Bioassay for Pesticides

## NEPHAR 302 Pharmacognosy I Laboratory

**Program**

| Weeks | | Subject |
|---|---|---|
| A | | **DEMONSTRATION** for Microscopy Lab. |
| 1 | **MICROSCOPY** | **Ergastic Cell Compounds-1:** Starchs<br>**Ergastic Cell Compounds-2:** Crystals |
| 2 | | **Nonglandular Trichomes** |
| 3 | | **Secretory Tissues (Glandular Tissues) and Stomata**<br>**Sclereids**<br>***Flores, Cortex, Radix Elements***<br>***Pollens*** |
| B. | | **DEMONSTRATION** for PLANT CHEMISTRY (PHYTOCHEMISTRY) |
| 4 | **PHYTOCHEMISTRY** | **Qualitative and Quantitative Analysis of lipids**<br>**Identification of Fatty Oils by Thin-Layer Chromatography**<br>**EUROPEAN PHARMACOPEIA 6.0** |
| 5 | | **TLC Analysis of Natural Products: Pigments**<br>**Flavonoids, Anthocyanins, Betalains (Betacyanins) and other Pigments (Crocins)** |
| 6 | | **TLC Analysis of Natural Products**<br>**PHENOLS: Coumarins**<br>**DIARYLHEPTANOIDS: Curcumins**<br>**Naphthodianthrones**: Hypericin<br>**Anthraquinones** |
| C | | RESULTS & DISCUSSION |

## B. Methods in Pharmacognostical Analysis: Microscopical Studies

| A. | **MICROSCOPY** |
|---|---|
| | **Demonstration for Microscopical Studies**<br>General Rules in Microscopical studies<br>Microscope Parts<br>Uses of Microscope<br>Reagents used in Microscopical Studies for dying cell wall |
| 1. | **Ergastic Cell Compounds-1**<br>Starchs (Tritici amylum, Solani amylum, Maydis amylum, Oryzae amylum)<br><br>**Ergastic Cell Compounds-2**<br>Crystals: **Simple crystals, twin crystals** (Hyoscyami folium)<br>**Crystal sand** (Cinchona cortex)<br>**Druse crystals** (Rhei rhizome)<br>**Raphide crystals** (Scillae bulbus) |
| 2. | **Nonglandular Trichomes**<br>**Multicellular trichomes** (Thymi folium)<br>**Unicellular trichomes** (Malva folium)<br>**Unicellular trichomes** (Melissa folium)<br>**Stellate trichomes** (Rosmarini folium)<br>**Stalked Stellate (star-shaped) trichomes** (Malva sylvestris flos)<br>**T-shaped trichomes** (Absinthii herba)<br>**Branched trichomes** (Lavandulae flos) |
| 3. | **Secretory Tissues (Glandular Tissues) and Stomata**<br>**Glandular trichomes** and **stomata** in Lamiaceae (Menthae folium)<br>**Schizogenous Oil Glands** and **Stomata** in Myrtaceae (Eucalyptus folium)<br>**Secretory Canals** in Apiaceae (Anisi fructus)<br><br>**Sclereids**<br>**Stone cells** (Cinnamomi cassiae cortex)<br>**Idioblasts** (Theae folium),<br>**astrosclereids** (*Nuphar, Nymphaea* spec.) |
| | **Flores, Cortex, Radix Elements**<br>**Flores**: Pollen, Stigma, Endotecium cells (**Chamomillae flos**)<br>**Cortex**: Sclerenchyma, cork, parenchyma (**Cinchona cortex**)<br>**Radix**: Sclerenchyma, cork, parenchyma, xylem (**Liquiritiae radix**)<br><br>**Further Examples for Pollens**<br>Malvae folium, Malvae flos<br>Lavandulae flos<br>Helichrysi flos<br>Croci stigma |

## GENERAL RULES IN MICROSCOPICAL STUDIES (Important):

1. Before examining your samples you should clean your microscobe, arrange the mirrors.
2. Examine the organoleptic specifications of the sample and write down for your report.
3. Prepare your samples carefully as described.
4. Prepare your samples with Sartur R. first and write down the colourings of the sample.
5. Prepare your samples with cloralhydrate and draw the cells, tissues etc. for your report
6. Write down the scales you use.
7. After drawing the element you see, you need to get approval for your drawings.
8. When you work with unknown samples you should note the code on your sample and need to use your lab guidebook.
9. You should write your analysis as a report to your lab book
10. When you work with powdered samples you need to keep your cachets closed and keep clean the needles you use all the time. You should make sure that the reactive bottles are closed properly after you use.
11. You need to clean your microscope and bench before you leave the laboratory.

**What you should bring during the Pharmacognosy Laboratory I**

i. White coat.
ii. Cleaning tissues.
iii. Matches.
iv. This manual.
v. For Microscopical and Phytochemical laboratory studies, the separate report **form*** for distinct studies has been prepared. They can be found as the last pages of this manual.

***Students should replicate these forms for their own work. Thus, they can keep their guide in hand to study the following laboratory works.**

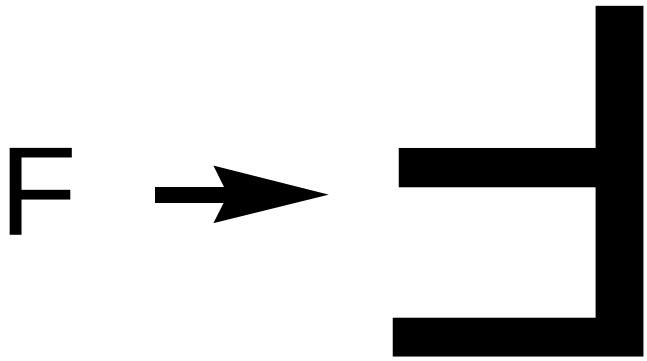
| 1st week | Date: |
|---|---|
| **Microscopic Studies**<br>**Microscope usage** | |
| Sample: **Letters cut out from a newspaper** | |

Analysis:

1. Set your microscope to a flat surface.
2. Set your microscopes mirror or light, diaphragm and condanser.
3. Set your letter sample between slide and lamel and place on the stage.
4. Engage the 10x objective in the light path.
5. Bring the specimen (letter) in focus.

Examine your letter sample with the smallest objective. Note the apparance is inversed or not.

The letter placed on the microscope, compare what you see. Note the differences. This will be helpful in the next microscopic studies in pulverized drugs analysis.



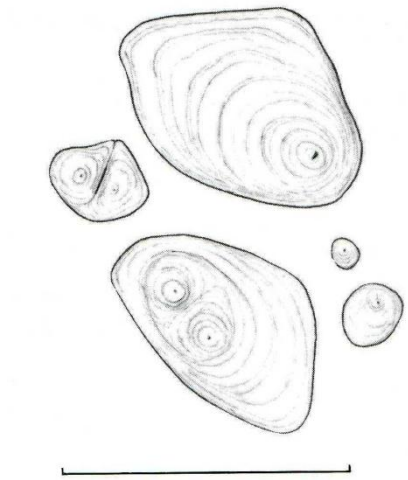
**For future studies with pulverized drugs, if you use Chloral Hydrate or Sartur reagents, you have to heat your preparate gently over a flame to clarify and to remove the air bulbs.**

**Questions**:

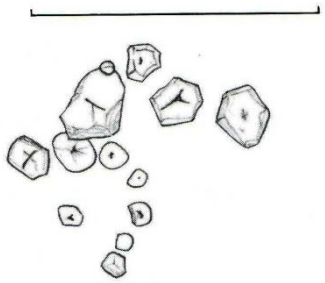
1. Describe the mechanical and optical parts of a microscope.
2. If you use 15x ocular lens and 40:1 objective how you calculate the magnification scale for your microscope?

| 1st week | Date: |
|---|---|
| **Ergastic Cell Compounds-1** | |
| Sample: **Amylum Drugs, Starches** | |

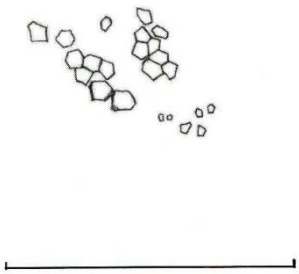
**Starch**: Prepare your sample with Sartur R. and note the colour of starch molecules. Prepare your sample with distilled water and draw the shapes of starch molecules



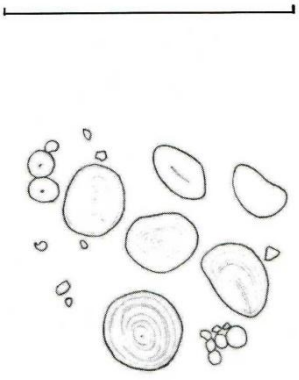
**Solani amylum**



**Maydis amylum**



**Oryzae amylum**



**Tritici amylum**

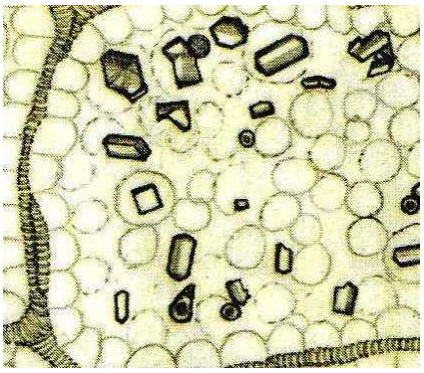
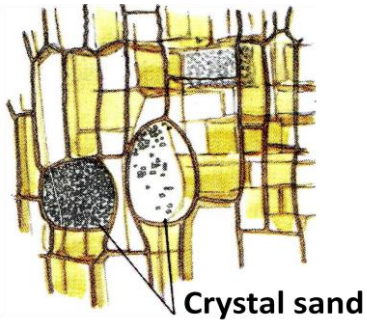
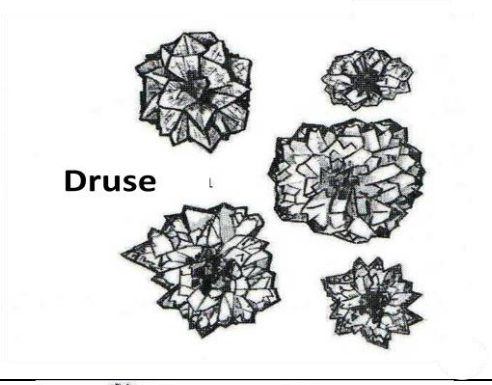
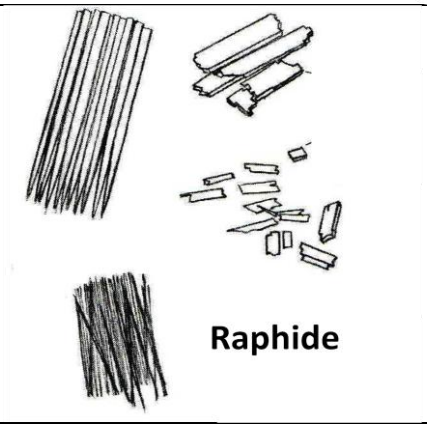
100 µm

Questions:

1. Describe the starch and explain how a starch molecule occurs.
2. What would happen if you heat starch with water?
3. Can you examine starch with Chloral hydrate R.?
4. Describe the chemical structure of starches.

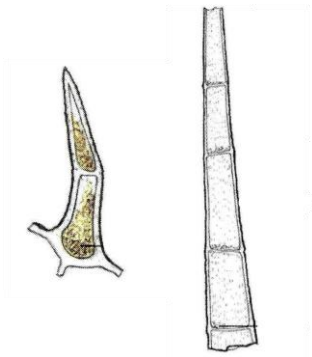
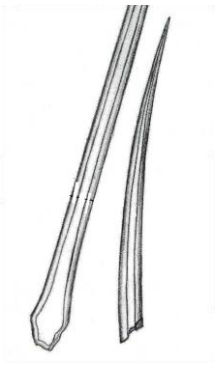
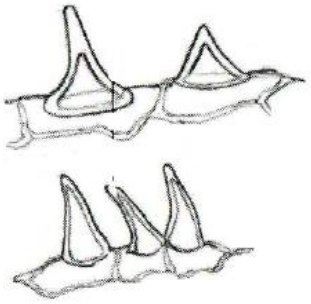
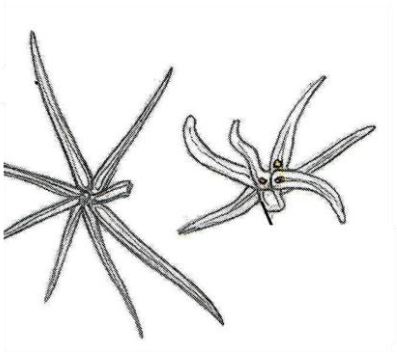
| 1st Week | Date: |
|---|---|
| **Ergastic Cell Compounds-2** | |
| Sample: **Crystals** (Calcium oxzalate and Calcium Carbonate crystals) containing drugs | |

**Crystals:** Prepare your sample with Chloral hydrate Reagent.

| | |
|---|---|
| **Simple crystals**<br><br>Drug : Hyoscyami folium (Folia Hyoscyami)<br>*Plant* : *Hyoscyamus niger,* Banotu<br>Family : Solanaceae<br>***Reagent***: *Chloral hydrate* |  |
| **Crystal sand**<br><br>Drug : Cinchona cortex (Cortex Chinae)<br>*Plant* : *Cinchona pubescens,* Kına kına<br>Family : Rubiaceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **Druse crystals**<br><br>Drug : Rhei radix (Rhizoma Rhei)<br>*Plant* : *Rheum palmatum*, Ravent<br>Family : Polygonaceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **Raphide crystals**<br><br>Drug : Scillae bulbus (Bulbus Scillae)<br>*Plant* : *Urginea maritima, Adasoğanı*<br>Family : Liliacae (= Alliaceae)<br><br>***Reagent***: *Chloral hydrate* |  |

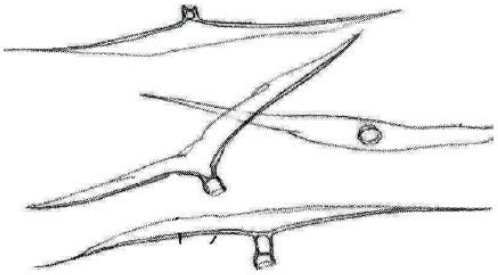
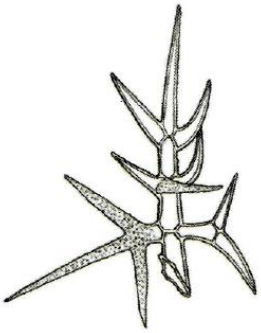
| 2nd Week | Date: |
|---|---|
| **Nonglandular Trichomes – 1/2** | |
| Samples: **Folium Drugs** (Leaf drugs) with uni- and multicellular, branched, stellate, T-shaped trichomes | |

**Nonglandular Trichomes:** Prepare your samples with Chloral hydrate Reagent.

| | |
|---|---|
| **<u>Multicellular trichomes</u>**<br><br>Drug : Thymi folium<br>*Plant* : *Thymus serpyllum,* **Kekik**<br>Family : Lamiaceae<br>***Reagent***: *Chloral hydrate* |  |
| **<u>Unicellular trichomes</u>**<br><br>Drug : Malva folium<br>*Plant* : *Malva sylvestris,* **Ebegümeci**<br>Family : Malvaceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **<u>Unicellular trichomes</u>**<br><br>Drug : Melissa folium<br>*Plant* : *Melissa officinalis,* **Oğulotu**<br>Family : Lamiaceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **<u>Stellate trichomes (Star shaped trichomes)</u>**<br><br>Drug : Rosmarini folium<br>*Plant* : *Rosmarinus officinalis*, **Biberiye**<br>Family : Lamiaceae<br><br>***Reagent***: *Chloral hydrate* |  |

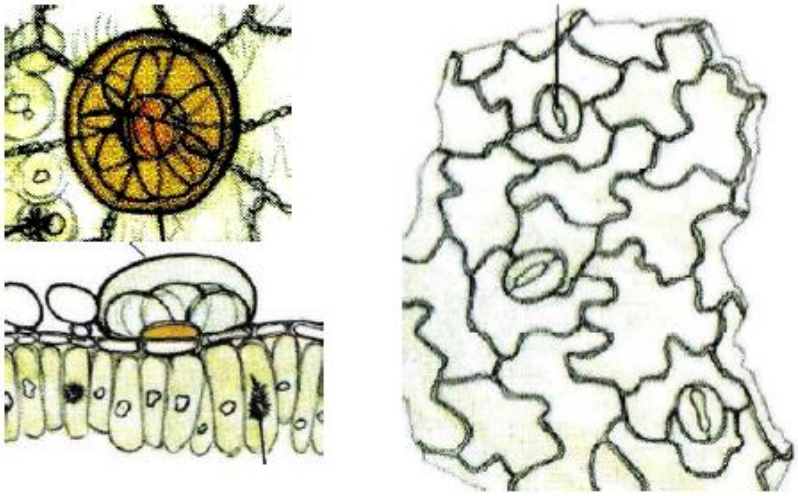
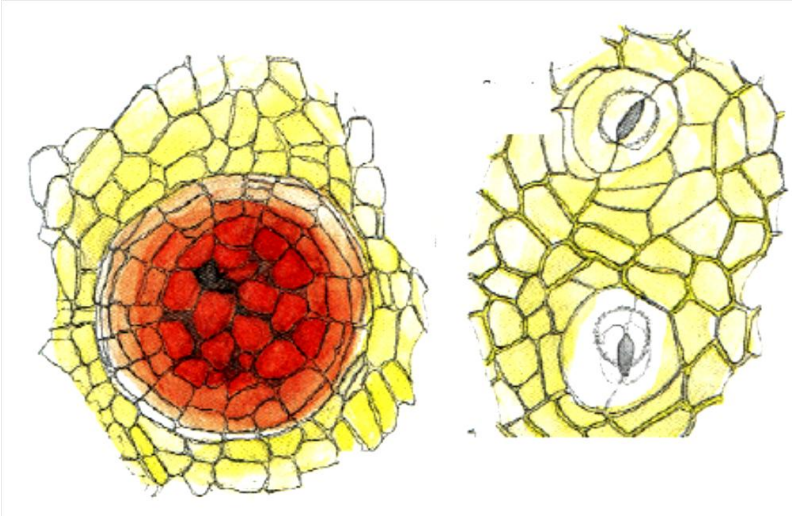
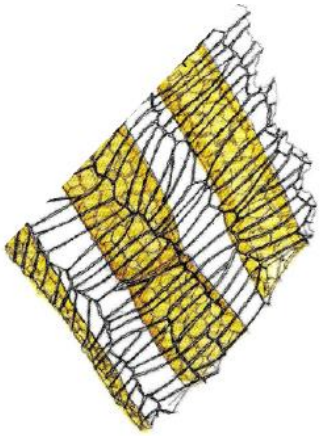
| 2nd Week | Date: |
|---|---|
| **Nonglandular Trichomes – 2/2** | |
| Samples: **Folium Drugs** (Leaf drugs) with uni- and multicellular, branched, stellate, T-shaped trichomes | |

**Nonglandular Trichomes:** Prepare your samples with Chloral hydrate Reagent.

| | |
|---|---|
| **<u>Stalked Stellate (star) trichomes</u>**<br><br>Drug : Malvae sylvestris flos<br>*Plant* : *Malva sylvestris,* **Ebegümeci**<br>Family : Malvaceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **<u>T-shaped trichomes</u>**<br><br>Drug : Absinthi herba<br>*Plant* : *Artemisia absinthium,* **Pelin Otu**<br>Family : Asteraceae<br><br>***Reagent***: *Chloral hydrate* |  |
| **<u>Branched trichomes</u>**<br><br>Drug : Lavandulae flos<br>*Plant* : *Lavandula angustifolia,* **Lavanta**<br>Family : Lamiaceae<br><br>***Reagent***: *Chloral hydrate* |  |

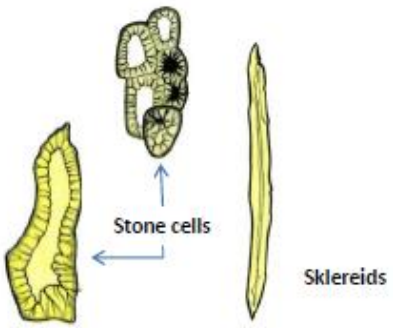
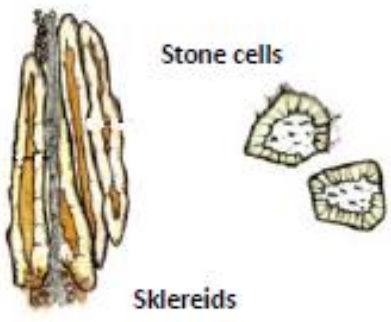
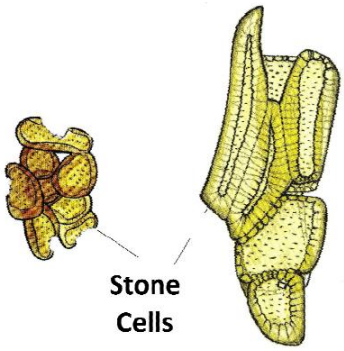
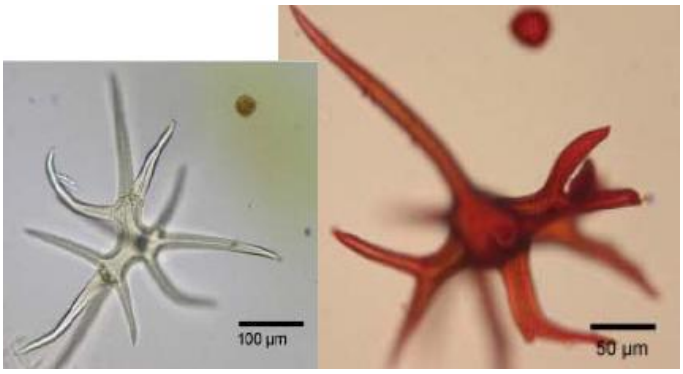
| 3rd Week | Date: |
|---|---|
| **Stomata and glandular tissues (Secretory Tissues) – 1/2** | |
| Samples: **Folium** and **Fructus** Elements | |

**Stomata and Secretory Tissues:** Prepare your samples with **Sartur** Reagent.

| | |
|---|---|
| **<u>Glandular trichomes and stomata</u>**<br><br>Drug : Mentha folium<br>*Plant* : *Mentha piperita*<br>Family : Lamiaceae (Labiatae)<br><br>***Reagent***: *Sartur R.* |  |
| **<u>Schizogenous oil glands and stomata</u>**<br><br>Drug : Eucalyptus folium<br>*Plant* : *Eucalyptus globulus*<br>Family : Myrtaceae<br><br>***Reagent***: *Sartur R.* |  |
| **<u>Secretory canals</u>**<br><br>Drug : Anisi fructus<br>*Plant* : *Pimpinella anisum*<br>Family : Apiaceae (Umbelliferae)<br><br>***Reagent***: *Sartur R.* |  |

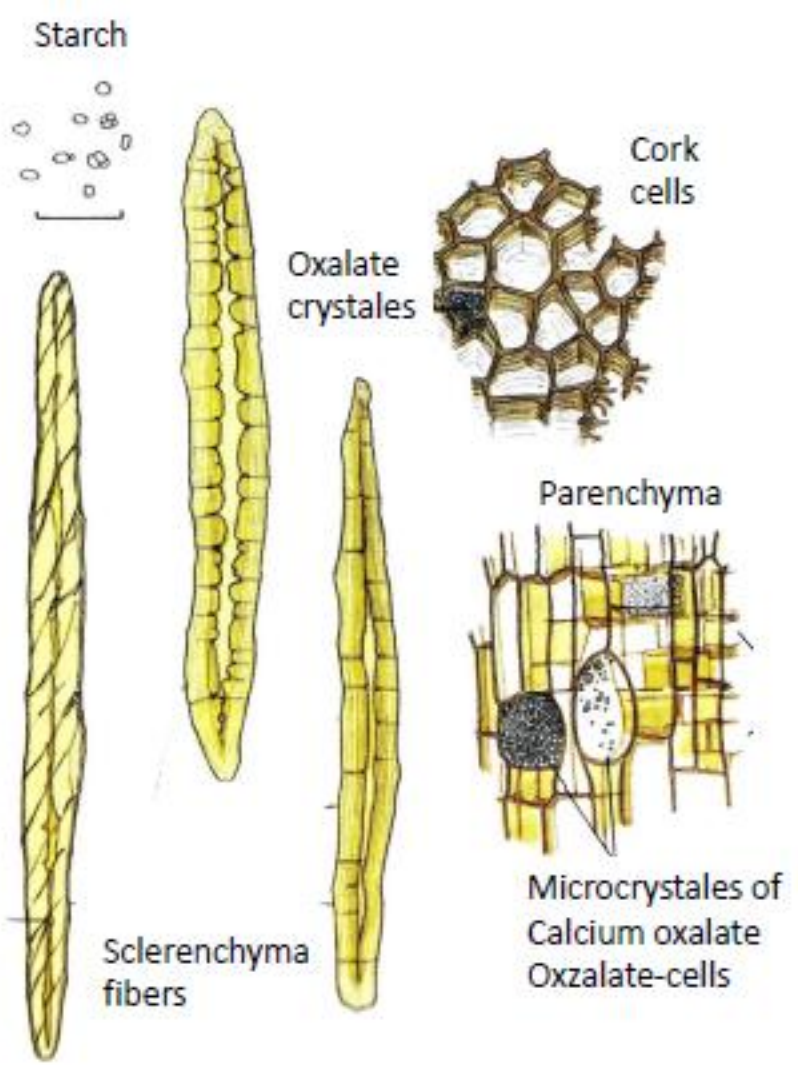
| 3rd Week | Date: |
|---|---|
| **<u>Sclereids:</u> Schlerenchyma, Stone cells, Idioblasts – 2/2** | |
| Samples: **Cortex, Flos** and **Folium** Drugs | |

**Sclereids:** Prepare your samples with **Sartur** Reagent.

| | |
|---|---|
| **<u>Sclereids</u>**<br>**Stone cells**<br><br>Drug : Cinnamomi cortex<br>*Plant* : *Cinnamomum zeylanicum,* **Tarçın**<br>Family : Lauraceae<br><br>***Reagent***: *Sartur R.* |  |
| **<u>Sclereids</u>**<br>**Stone cells**<br><br>Drug : Caryophylli flos<br>*Plant* : *Syzygium aromaticum,* **Karanfil**<br>Family : Myrtaceae<br><br>***Reagent***: *Sartur R.* |  |
| **<u>Sclereids</u>**<br>**Stone cells**<br><br>Drug : Gallae, **Mazı**<br>*Plant* : *Quercus infectoria,* **Meşe**<br>Family : Fagaceae<br><br>***Reagent***: *Sartur R.* |  |
| **<u>Sclereids</u>**<br>**Idioblast (Astrosclereids)**<br><br>*Plant* : *Nuphar spec., Nymphaea spec.** **Nilüfer**<br>Family : Nymphaceae<br><br>*R.R. Marrotte, G.L. Chmura, P.A. Stone, ***Review of Palaeobotany and Palynology* 169, 29–37 (2012)** |  |

| | Date: |
|---|---|
| **Cortex, Radix and Flores Drugs – 1/3** | |

| **Subject**: Cortex Elements | |
|---|---|
| **Drug:** Cinchona cortex<br>**Plant:** *Cinchona pubescens* (Syn. *C. succirubra*)<br>**Family:** Rubiaceae<br>**Reagent:** Chloral hydrate, Sartur<br>**Scale:** (the scale of objective) x (the scale of ocular) | **<u>Organoleptic properties</u>**<br>**Colour:**<br>**Odour:**<br>**Taste:**<br>**Appearance:** |



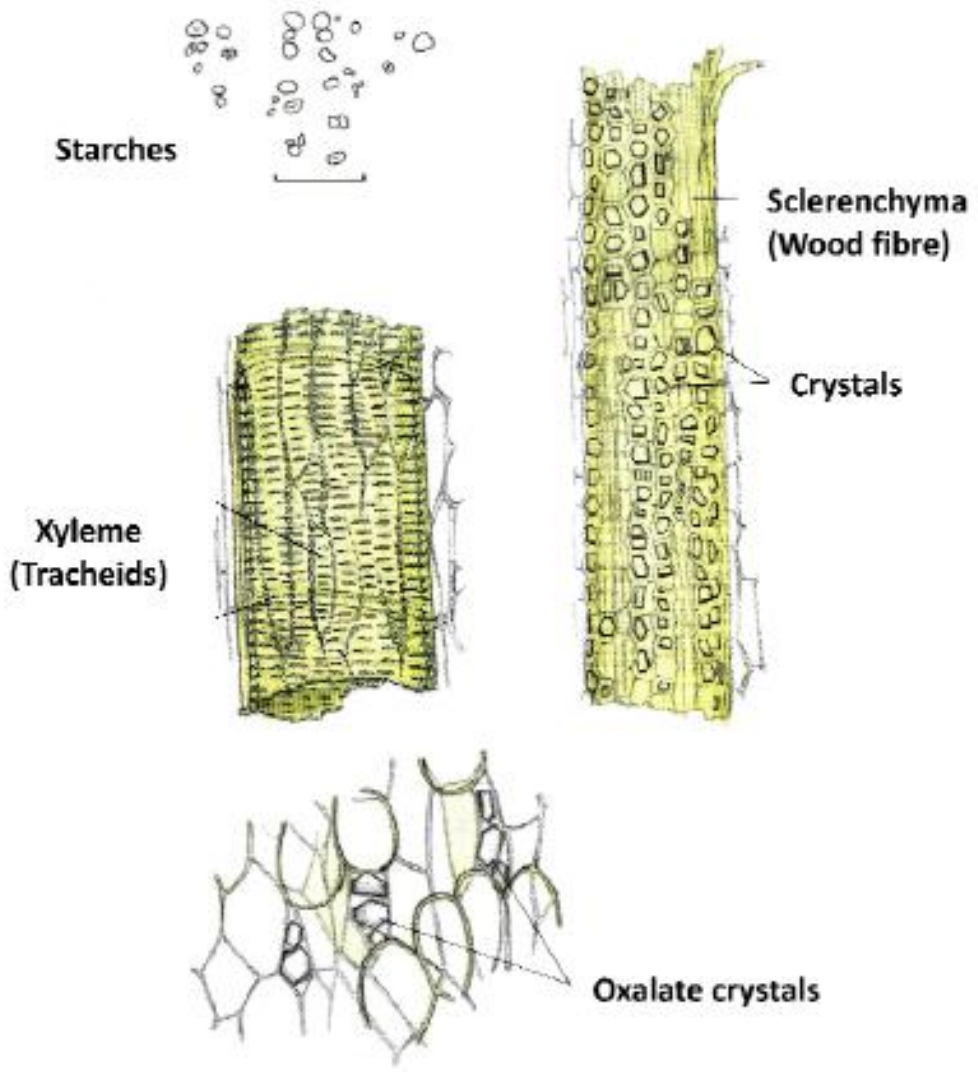

**Observations:** Cork cells are brown, Sclerenchyme fibers are yellow

Drawings from **Pulver – Atlas der Drogen – der deutschsprachigen Arzneibücher**
Walter Eschrich, Deutscher Apotheker Verlag, Stuttgart, 2009

| | Date: |
|---|---|
| **Cortex, Radix and Flores Drugs – 2/3** | |

**Subject**: Radix Elements

| | |
|---|---|
| **Drug:** Liquiritiae radix<br>**Plant:** *Glycyrrhiza glabra*<br>**Family:** Fabaceae<br>**Reagent:** Chloral hydrate, Sartur<br>**Scale:** (the scale of objective) x (the scale of ocular) | **<u>Organoleptic properties</u>**<br>**Colour:** Yellow<br>**Odour:** None<br>**Taste:** Sweet at the beginning and then sourish<br>**Appearance:** Heterogeneous |



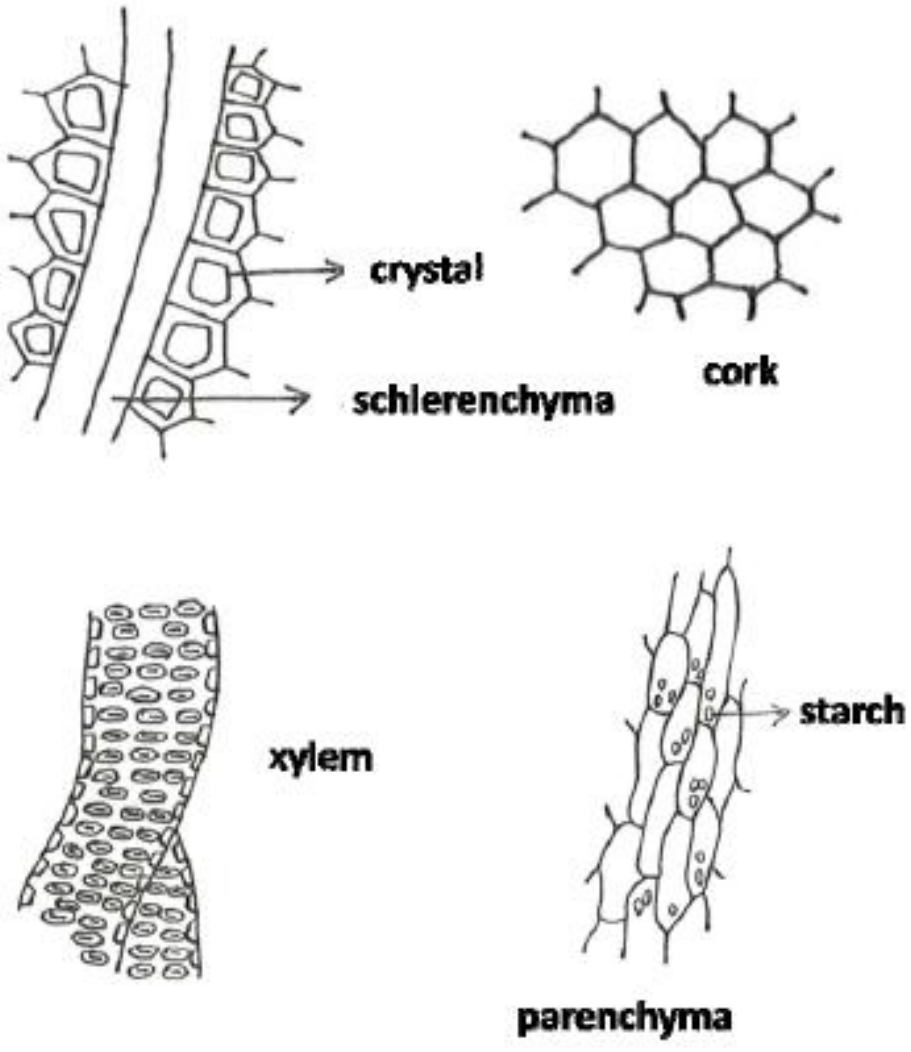

**Observations:** Yellow sclerenchyma tissues, Orange-brown cork tissues and blue-purple starch were observed.

Drawings from **Pulver – Atlas der Drogen – der deutschsprachigen Arzneibücher**
Walter Eschrich, Deutscher Apotheker Verlag, Stuttgart, 2009

| | Date: |
|---|---|
| **Cortex, Radix and Flores Drugs – 2/3** | |

| **Subject**: Radix Elements | |
|---|---|
| **Drug:** Liquiritiae radix<br>**Plant:** *Glycyrrhiza glabra*<br>**Family:** Fabaceae (Leguminosae)<br>**Reagent:** Chloral hydrate, Sartur Reagent<br>**Scale:** (the scale of objective) x (the scale of ocular) | **Organoleptic properties**<br>**Colour:** Yellow<br>**Odour:** None<br>**Taste:** Sweet at the beginning and then sourish<br>**Appearance:** Heterogeneous |

**Drawings**

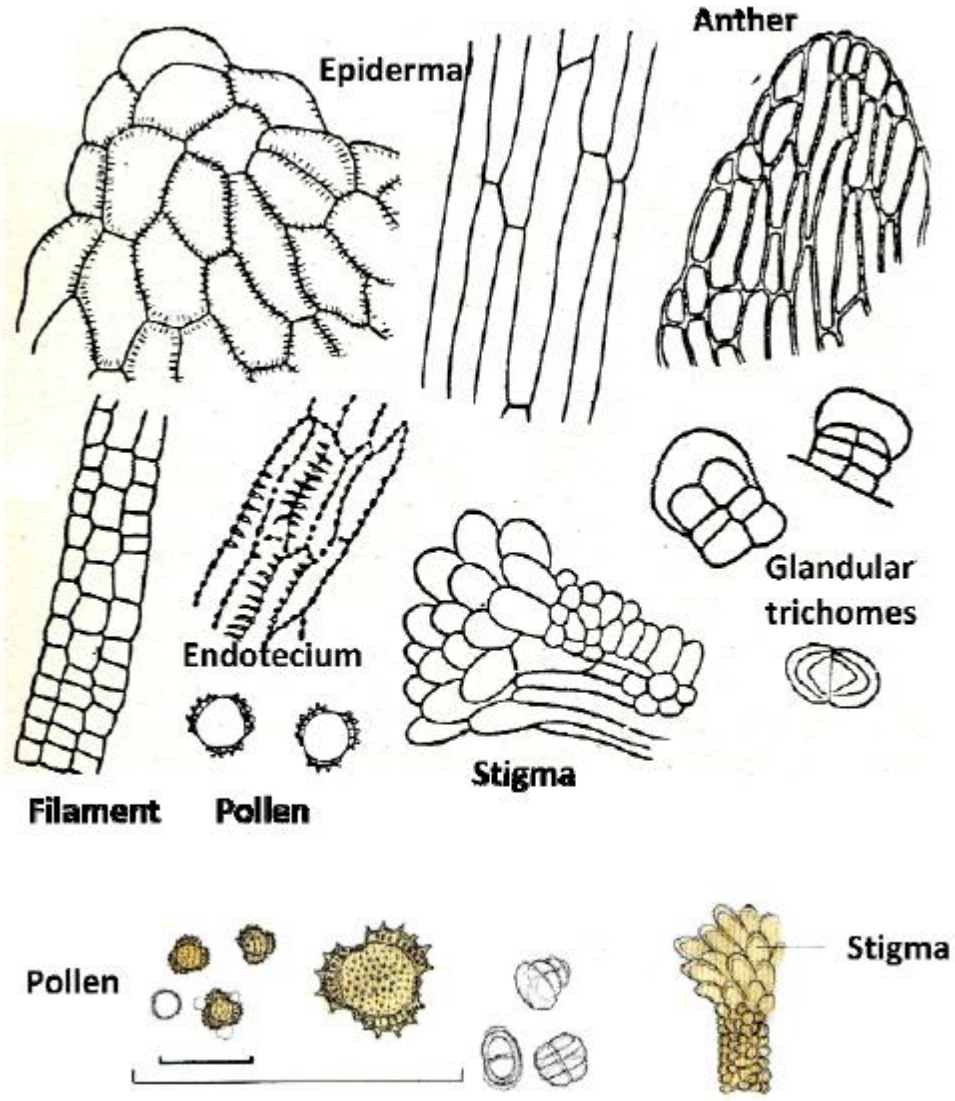



**Observations:** Yellow sclerenchyma tissues, Orange-brown cork tissues and blue-purple starch were described

| | Date: |
|---|---|
| **Cortex, Radix and Flores Drugs – 3/3** | |

**Subject**: Flores Elements

| | |
|---|---|
| **Drug:** Chamomillae vulgaris flores<br>**Plant:** *Chamomillae vulgaris*<br>**Family:** Asteraceae<br>**Reagent:** Sartur R.<br>**Scale:** (the scale of objective) x (the scale of ocular) | **<u>Organoleptic properties</u>**<br>**Colour:**<br>**Odour:**<br>**Taste:**<br>**Appearance:** |

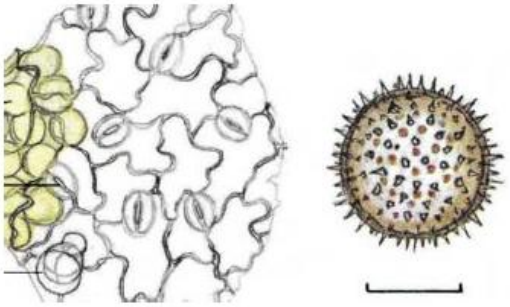
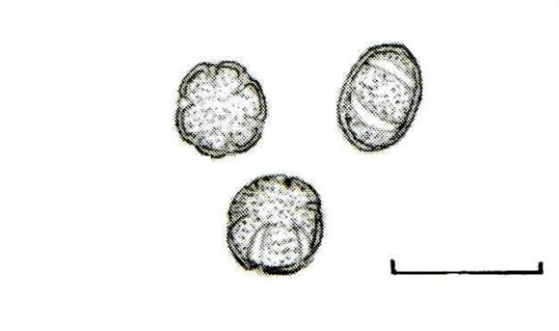
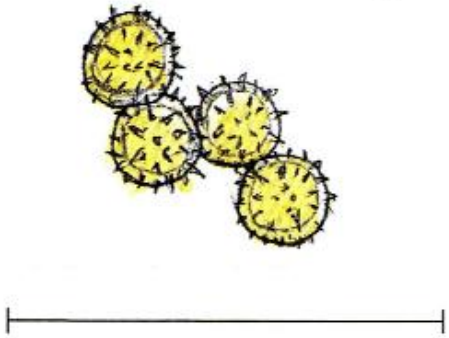
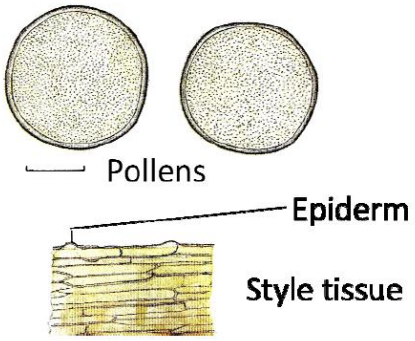



**Observations:**

Drawings from **Bitkisel Drogların Anatomik Yapısı**
Asuman BAYTOP, İstanbul Üniversitesi Eczacılık Fakültesi Yayınları, İstanbul, 1987

| | Date: |
|---|---|
| **Further Examples for Pollens** | |
| Samples: **Flos** Drugs | |

**Pollen:** Prepare your samples with **Chloralhydrate Reagent**.

| | |
|---|---|
| **Pollen**<br><br>Drug : Malvae folium, Malvae flos<br>*Plant* : *Malva sylvestris,* Mallow, ***Ebegümeci***<br>Family : *Malvaceae*<br><br>***Reagent***: *Chloralhydrate R.* |  |
| **Pollen**<br><br>Drug : Lavandulae flos<br>*Plant* : *Lavandula angustifolia,* Levander, ***Lavanta***<br>Family : Lamiaceae<br><br>***Reagent***: *Chloralhydrate R.* |  |
| **Pollen**<br><br>Drug : Helichrysi flos<br>*Plant* : *Helichrysum arenarium,* Immortelle **Altın çiçek, Ölmez çiçek**<br>Family : Asteraceae<br><br>***Reagent***: *Chloralhydrate R.* |  |
| **Pollen**<br><br>Drug : Croci stigma<br>*Plant* : *Crocus sativus*, Saffron, **Safran**<br>Family : Iridaceae<br><br>***Reagent***: *Chloralhydrate R.* |  |

P.S.: *Copy of next page will be used for microscopy studies. It should be produced for each case.*

| **NEPHAR 302 PHARMACOGNOSY I LABORATORY** | **Microscopy Lab. No:** |
|---|---|
| **Name Surname :**<br>**Number :** | **Microscope No :**<br>**Assistant :** |
| **Subject**: | |
| **Drug:**<br>**Plant:**<br>**Family:**<br>**Reagent:**<br>**Scale:** | **<u>Organoleptic properties</u>**<br>**Colour:**<br>**Odour:**<br>**Taste:**<br>**Appearance:** |
| **Drawings:** | |
| Observations: | |

*Form A*

## C. Methods in Pharmacognostical Analysis:

**Phytochemical Analysis (PHYTOCHEMISTRY)**

In this second part of the laboratory studies, extraction and the application of chromatographical (Thin Layer Chromatography = TLC; Column Chromatography = CC) methods for detection and isolation of natural compounds (primary and secondary plant metabolites) will be studied.

**Plant Metabolites**

| | |
|---|---|
| **Pharmacognosy I** | **Compounds of Primary Metabolism**<br>**CARBOHYDRATES**<br>**LIPIDS**<br>**AMINO ACIDS, PEPTIDES, PROTEINS AND ENZYMES**<br><br>**Compounds of Secondary Metabolism**<br>**PHENOLICS**<br>**Shikimates:**<br>**PHENOLS AND PHENOLIC ACIDS**<br>PHENYLPROPANOIDS<br>**COUMARINS**<br>Lignans<br>PHENYLPROPANOIDS elongated in Chain<br>**DIARYLHEPTANOIDS**<br>ARYLALKANONES<br>STILBENOIDS<br>XANTHONES<br>STRYLPYRONES<br>**FLAVONOIDS**<br>**ANTHOCYANINS (and BETACYANINS)**<br>Tannins<br>**Acetates (Polyketides):**<br>**QUINONES**<br>Simple Quinones<br>**NAPHTHOQUINONES**<br>**ANTHRAQUINONES** |
| **Pharmacognosy II** | **TERPENOIDS AND STEROIDS** |
| **Pharmacognosy III** | **ALKALOIDS** |

**Terpenoid&Steroid and Alkaloid** containing plants and drugs are the subject of **pharmacognosy II and III.**

From the metabolites listed above, assay for **LIPID, COUMARIN, FLAVONOID, ANTHOCYANIN, BETACYANIN and QUINONE** (NAPHTHOQUINONE AND ANTHROQUINONE) from the selected plants and drugs will be applied as a subject of Pharmacognosy I laboratory studies.

## PROGRAM

**PLANT CHEMISTRY, PHYTOCHEMICAL ANALYSIS (PHYTOCHEMISTRY)**

| Week | Subjects |
|---|---|
| | **DEMONSTRATION** |
| **4** | **Lipids – Fatty Acids – Glycerides**<br><br>**Plant Lipids**<br>**Qualitative and Quantitative Analysis of lipids**<br>**Soxhlet Extraction**<br>Ricini semen, Lini semen, Oliven fructus etc.<br>**Identification of Fatty Oils by Thin-Layer Chromatography**<br>**EUROPEAN PHARMACOPEIA 6.0** |
| **5** | **TLC Analysis of Natural Products: Pigments**<br>**Flavonoids, Anthocyanins, Betalains (Betacyanins) and**<br>**other Pigments (Crocins)**<br><br>**Flavonoids**: Calendula flos, Juglandis folium, Betulae folium, Matricariae flos, Tiliae flos, Citri-& Aurantii pericarpium, Cardui mariae fructus<br>**Anthocyanins, Betalains (Betacyanins) and other Pigments (Crocins)**<br>Cyani flos, Hibisci flos, Malvae flos, Croci stigma |
| **6** | **TLC Analysis of of Natural Products**<br><br>**PHENOLS**<br><br>**Coumarins:**<br><br>Pimpinella radix, Heraclei radix, Angelicae radix, Rutae herba, Ammi fructus,<br><br>PHENYLPROPANOIDS elongated in Chain<br>**DIARYLHEPTANOIDS**<br>Curcuma Rhizoma (**Curcumin**)<br><br>**Anthraquinones**<br><br>Aloe (**Aloin**), Rhei radix (**Rhein**), Sennae folium (**Sennosides A&B**) |

# LIPIDS – FATTY ACIDS – GLYCERIDES

## PLANT LIPIDS

**Pharmacognosy, Phytochemistry, Medicinal Plants**
J. Bruneton, Lavoisier Publishing, London New York, 1999
**Fundamentals of Pharmacognosy and Phytotherapy**
M. Heinrich, J. Barns, S. Gibbons, E. M. Williamson, Churchill Livingstone, London 2004
**European Pharmacopoeia 6.0**
**http://www. Cyperlipid.org/extract/**

Lipids are natural substances, esters of fatty acids and alcohols or polyols. They are constituents of cell structures such as membrane phospho-, and glycolipids, coating elements such as waxes or cutins, and also reserve substances and sources of energy for the cell.

**Simple lipids:** Esters of a fatty acid and an alcohol
Glycerol, constituents of triacylglycerols or triglycerides
A high molecular-weight aliphatic alcohol, a constituent of waxy esters.
**Complex lipids**: Phospho-, and glycolipids

### TRIGLYCERIDES (Simple Lipids)

**Triacylglycerols** are practically absent in vegetative organs (leaves). They are stored as oily inclusions called oleosomes, which arise from the endoplasmic reticulum, and at times gather in large piles in the cells of reserve tissues; these are seeds.

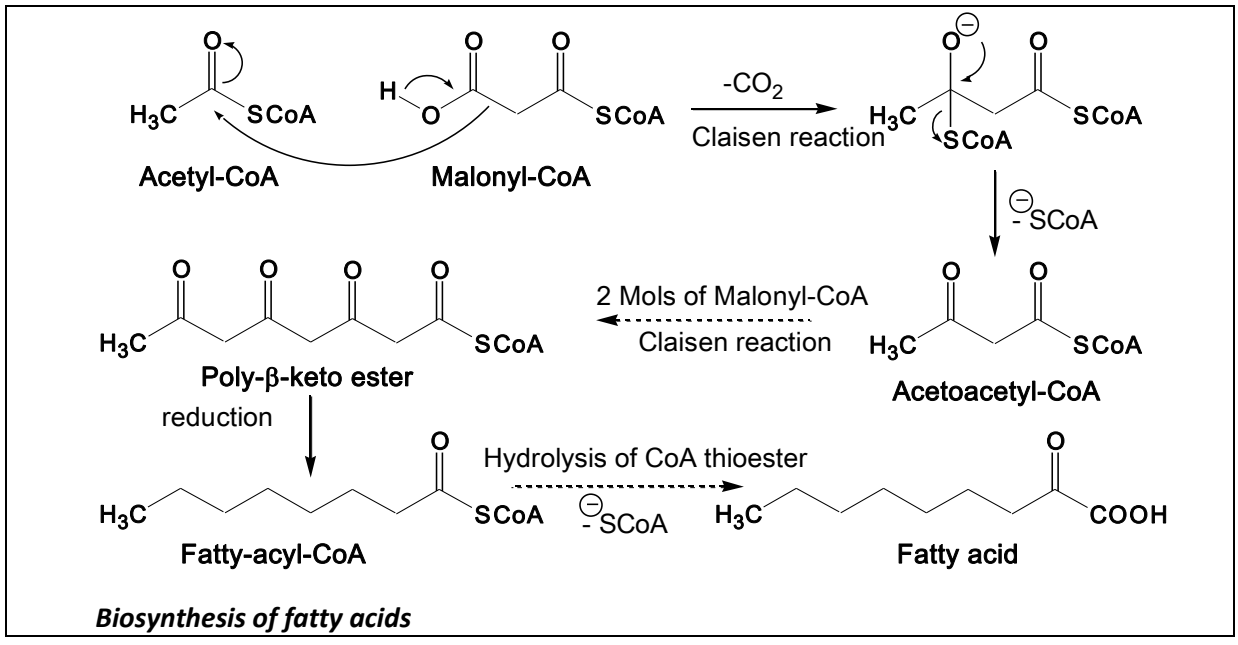
Their structures are triesters of a triol, glycerol, and of fatty acids, in other words aliphatic carboxylic acids of variable length which have an even number of carbon atoms.

Fatty acids are biosynthetically polyketid derivatives.

### *THE POLYKETIDS*

*Polyketides comprise many antibiotics (macrolides and tetracyclines),* ***fatty acids*** *and aromatic compounds (anthrone glycosides and anthracyclic antitumour agents).*

*They are mainly acetate (C2) derived metabolites and occur throughout all organisms (as* ***fatty acids*** *and* ***glycerides****).*




***Biosynthesis of fatty acids***

**Biosynthesis**

The biosynthesis of these compounds begins with the condensation of of one molecule of malonyl-CoA with one molecule of acetyl-CoA to form a simple polyketide acetoacetyl-CoA. Further condensation reactions between another molecule of malonyl-CoA and the growing polyketide lead to chain elongation, in which every other carbon in the chain is a carbonyl group. These chains are known as poly-β-keto esters and are the reactive intermediates that form the polyketides. Using these esters, large chains such as fatty acids can be constructed. Reduction of the carbonyl groups and hydrolysis of the –SCoA thioester leads to the fatty acid class of compounds.

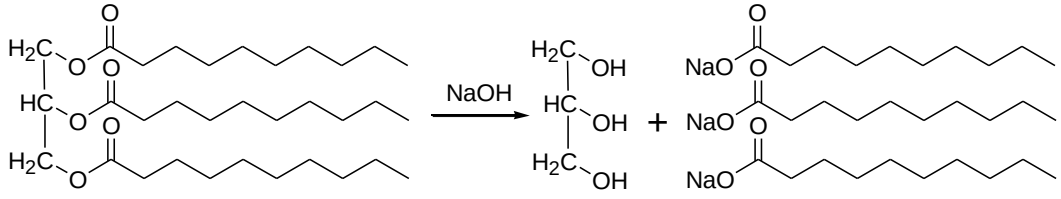
**FATTY ACIDS – GLYCERIDES**

This group of poyketides is widely distributed and present as a part of the general biochemistry of all organisms, particularly as components of cell memranes. They are ussually insoluble in water and soluble in organic solvents such as hexane, diethyl ether and chloroform.

These natural products are also referred to as fixed oils (liquid) or fats (solid), although these terms are imprecise as both fixed oils and fats contain mixtures of glycerides and free fatty acids and the state of the compound (e.g. liquid or solid) will depend on the temperature as well as composition.

Glycerides are fatty acid esters of glycerol (propane-1,2,3-triol). They are sometimes referred to as saponifiable natural products, meaning that they can be converted into soaps by a strong base (NaOH). The term saponifiable comes from the Latin word *sapo* meaning "soap". Saponification of fatty acisds and glycerides with sodium hydroxide results in the formation of the sodium salts of the fatty acids. The substituents (fatty acids) on glycerol unit may be same or different from each other.

Fatty acids are very important as formulation agents and vehicles in pharmacy and cosmetics. In Table 1, the most common fatty acids with their chemical formulae and sources are listed.




**Glyceride** Triglyceride of capric acid | **Glycerol** (Propane-1,2,3-triol) | **Sodium caprate** (3 Molecules)

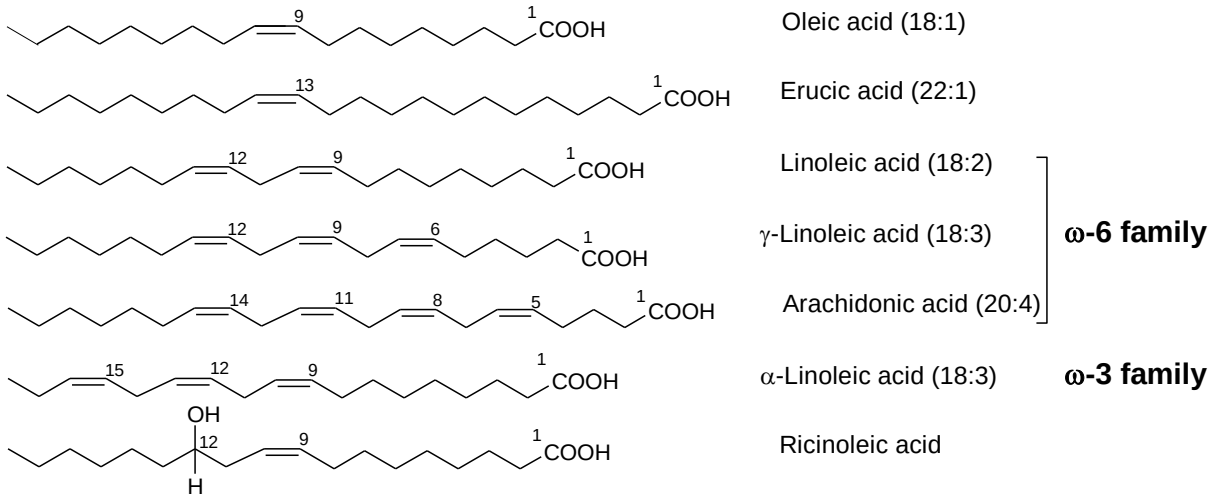
***Saponification of fatty acids and glycerides with NaOH***

**The saturated fatty acids** are widespread in nature. The three most common fatty acids, **myristic**, **palmitic** and **stearic acids** contain no double bonds.

**The unsaturated fatty acids** contain a varying number of double bonds. This, together with the lenght of the carbon chain, is indicated after the name of fatty acid. **Oleic acid** (18:1), which is widespread in plants and is a major compound component of olive oil, has an 18-carbon chain and one double bond. **α-Linoleic acid** (18:3, *Linum usitatissimum*) and **γ-Linoleic acid** (18:3, *Oenanthera biennis*) are the valuable unsaturated fatty acids. The latter is an essential fatty acid and is precursor to the prostoglandins, which are involved in many biochemical pathways. **Ricinoleic acid** is the main purgative ingredient of castor from the seed of *Ricinus communis*. The polyunsaturated fatty acids contain three or more double bonds and are particularly beneficial in the diet as antioxidants. The fish liver oils from cod and halibut are rich in polyunsaturated fatty acids.

| Table 1 | COMMON FATTY ACIDS | | |
|---|---|---|---|
| **Common name** | **Formula** | **Oil and Source** | Plant name (Family) or **animal** |
| **Butyric** | $CH_3(CH_2)_2COOH$ (4:0) | **Butter fat** | **Bovus taurus**, cow |
| **Caproic** | $CH_3(CH_2)_4COOH$ (6:0) | Coconut oil | *Cocos nucifera* (Palmae) |
| **Caprylic** | $CH_3(CH_2)_6COOH$ (8:0) | Coconut oil | *Cocos nucifera* (Palmae) |
| **Capric** | $CH_3(CH_2)_8COOH$ (10:0) | | *Cuphea viscosissima* (Lythraceae) |
| **Lauric** | $CH_3(CH_2)_{10}COOH$ (12:0) | Coconut and palm kernel | *Cocos nucifera, Elaeis guinensis* (Arecaceae) |
| **Myristic** | $CH_3(CH_2)_{12}COOH$ (14:0) | Coconut and palm kernel | *Cocos nucifera, Elaeis guinensis* (Arecaceae) |
| **Palmitic** | $CH_3(CH_2)_{14}COOH$ (16:0) | Coconut and palm kernel | *Cocos nucifera, Elaeis guinensis* (Arecaceae) |
| **Stearic** | $CH_3(CH_2)_{16}COOH$ (18:0) | Olive oil | *Olea europea* (Oleaceae) |
| **Arachidic** | $CH_3(CH_2)_{18}COOH$ (20:0) | Peanut oil | *Arachis hypogea* (Fabaceae) |
| **Behenic** | $CH_3(CH_2)_{20}COOH$ (22:0) | Carnauba wax | *Copernicia cerifera* (Palmae) |
| | | | |
| **Linoleic** | $CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$ (18:2) all *cis* | Soybean, | *Glycine max* (Fabaceae) |
| **α-Linoleic** | $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$ | Linseed oil | *Linum usitatissimum* (Linaceae) |
| **γ-Linoleic** | $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_4COOH$ | Evening primrose oil | *Oenanthera biennis* (Onagraceae) |
| | | | |
| **Oleic** | $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ (18:1) ***cis*** | Olive oil | *Olea europea* (Oleaceae) |
| **Erucic** | $CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$ (22:6) **all *cis*** | Rapeseed oil | *Brassica napus* var. *oleifera* (Brassicaceae) |
| **Nervonic** | $CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$ (24:1) **all cis** | Honesty oil | *Lunaria annua* (Brassicaceae) |
| | | | |
| **Eicosahexanenoic** | $CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2COOH$ (20:6) **all *cis*** | **Cod liver oil and halibut liver oil** | **Cod fish, flatfish** |
| **Docosahexaenoic** | $CH_3CH_2(CH{=}CHCH_2)_6CH_2COOH$ (22:6) **all *cis*** | | |
| | | | |
| **Ricinoleic** | $CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOH$ ***cis*** | Castor oil | *Ricinus communis* (Euphorbiaceae) |

*The lenght of the carbon chain and the number of double bonds are indicated after the formula of fatty acid. For example, **oleic acid,** $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ (18:1).




***The formulae of some fatty acids***

**Structure of glycerol esters:** A triglyceride may be homogenous or heterogenous depending on whether the fatty acid units that esterify the three alcohol group of glycerol are identical or different. In general, triglycerides are heterogenous and a vegetable oil is a complex mixture of triesters. However, saturated fatty acids esterify preferentially the primary alcohol functions of glycerol.

**Oil Production:** Compression using pressure, extraction with organic solvents are the common methods in oil production. Before proceeding with the recovery of the oil from the vegetable organs, strict quality control of the starting material (e.g. absence of foreign matter and of deterioration) , preliminary procedures - washing the olives, delintering cotton, shelling peanuts - are required.

1. **Extraction by Expression**. Generally screw presses are used because the yield better than the older hydraulic presses: They operate at higher pressures and continuously. Prior to compression, oil seeds rich in proteins are subjected to heating at 90 ˚C, which frees the oil by bursting cell structures, but also coagulates the proteins. Most often a fast drying step follows.

2. **Extraction by Solvents**. This type of extraction is applicable to intact seeds as well as to seeds partially extracted by expression. The solvent, generally hexane (bp 65 ˚C), is added to the cleaned, hulled, and rough-milled seeds. An organic phase is recovered which is a solution of the oil in the solvent miscella, and also a solvent-soaked defatted meal. Industrial setups usually have a counter-current design. Oil recovery ranges from 95 to 99%.

**Refining the crude oil**. Crude oil may contain water, free fatty acids, lecithins, resins, pigments, sterols, substances with odors nd tastes. Therefore,the refining processes are used (***degumming*** to remove musilage, ***neutralization*** due to the presens of free fatty acids, ***bleaching*** and ***deodorizing***).

***Degumming***: The hot oil is hydrated, whereupon the colloids form a dense gel which separates from the lighter oil. The gel is discarded and the oil dried under vacuum.

***Neutralization***. The free fatty acids, which are always present in the crude oil, are neutralized by dilute NaOH, $Na_2CO_3$ or $NH_4OH$. The soap formed adsorbs part of the inpurities: coloring matter, phenols, sterols, wax esters, etc..

***Bleaching***. This is done by passing the oil through diatomaceous earth or activated charcoal.

***Deodorizing***. The aldehydes and ketones responsible for the unpleasant odor of crude oil are eliminated by injecting steam into the very hot oil under high vacuum.

**Quality Control for Lipid-Containing Drugs: Tests for Fixed Oils**.

**General**:

Verification of identity and determination of the fixed oil content are the main assay. Determination of the fatty acid composition is carried out on methyl esters obtained by methylation. In isothermal chromatography, fatty acid esters are identified by their equivalent chain length.

**Methods in Pharmacopoeia**:

The common assay in the pharmacopoieas are the following.

**Specific gravity**.

**The acid value $I_A$** is the number that expresses, in milligrams the quantity of KOH required to neutralise the free acids in 1 g of the substance (oil) (EP 6)

**The peroxide value $I_P$** is the number expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance (EP 6).

**The saponification value $I_{AS}$** is the number that expresses in milligrams the quantity of KOH required to neutralise the free acids and to saponify the esters present in 1 g of the substance (EP 6).

**The unsaponifiable matter**. These are mostly carotenoids, sterols, tocopherols, terpenoid alcohols, and hydrocarbons and they are not volatile at 100 ˚C.

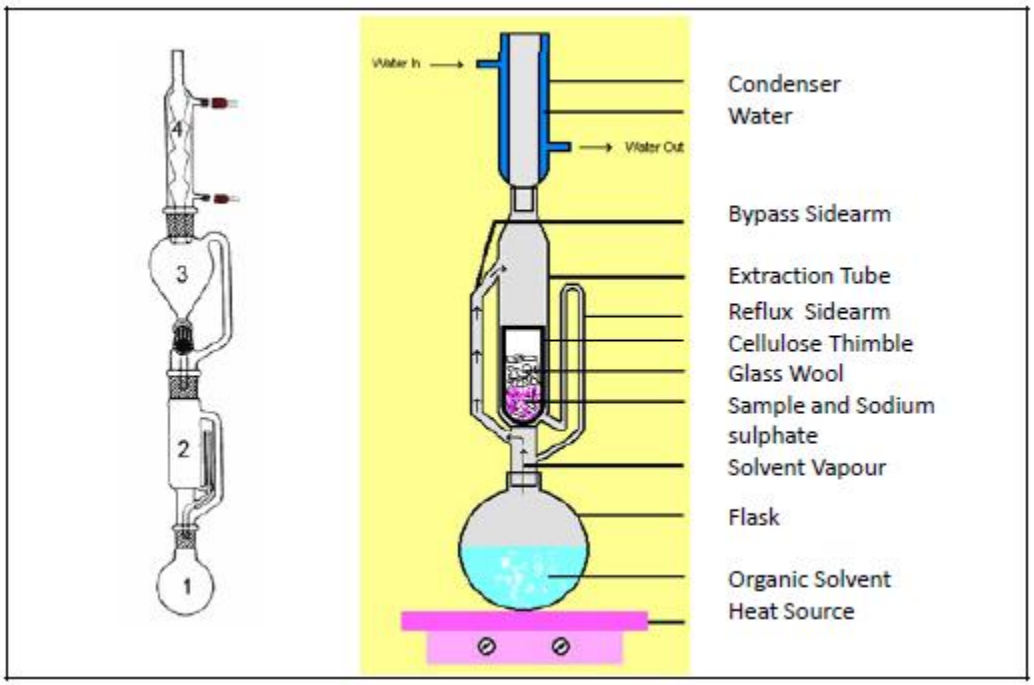
**Foreign oils in fixed oils**. This test may be done by TLC (**EP 6, Vol. 1, 2.3.2**, page 106). In the French Pharmacopoiae (10th Edition, V.3.3.5), TLC plates (Normal phase Silica gel coated) first developed in a petroleum ether solution of parafin are used.Test solutions consist of the mixture of fatty acids from saponification. Reference standart solution consist of the mixture of fatty acids from saponification of a 19:1 mixture of corn oil and rapeseed oil. After developing the chromatogram, the spots corresponding to fatty acids are visualized by iodine vapor.

**Alkaline inpurities.** Neutralization of an acetone solution of oil in the presence of bromothymol blue.

**Refractive index.**

# 6 EXTRACTION OF LIPIDS: *SOXHLET EXTRACTION*

The method described by Soxhlet in 1879 is the most commonly used example of a semi-continuous method applied to extraction of lipids from foods. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware.




In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber [**2**], which is suspended above a flask containing the solvent [**1**] and below a condenser [**4**]. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and lipid is removed. In some device a funnel [**3**] allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask [**2**] is then evaporated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated.

**ASSAY**: 10 g exactly weighed sample are mixed and pulverised with one of three parts (1/3) of 10 g anhidr sodium sulphate using a mortar. The mixture is transferred to a cellulose thimble of the Soxhlet aparey quantitatively using the rest of anhidr sodium sulphate. The content of the cellulose thimble is covered by glass wool (or defatted cotton). Flask is filled to half by an organic solvent such as hexane or dichloromethane and heated approximately 2 hours for the extraction. At the end of the extraction, after the last flush of the solvent from the extraction unit to the flask, lipid containing solvent is transferred to another flask weighed exactly. Slovent is removed using rotary evaporator under the vacuum. The residual solvent is removed by keeping the flask in a drying oven at 100 ˚C. Finally, flask is cooled in a desiccator until the constant weight. The difference between the dead load (tare) and gross weight of the flask gives the oil yield.

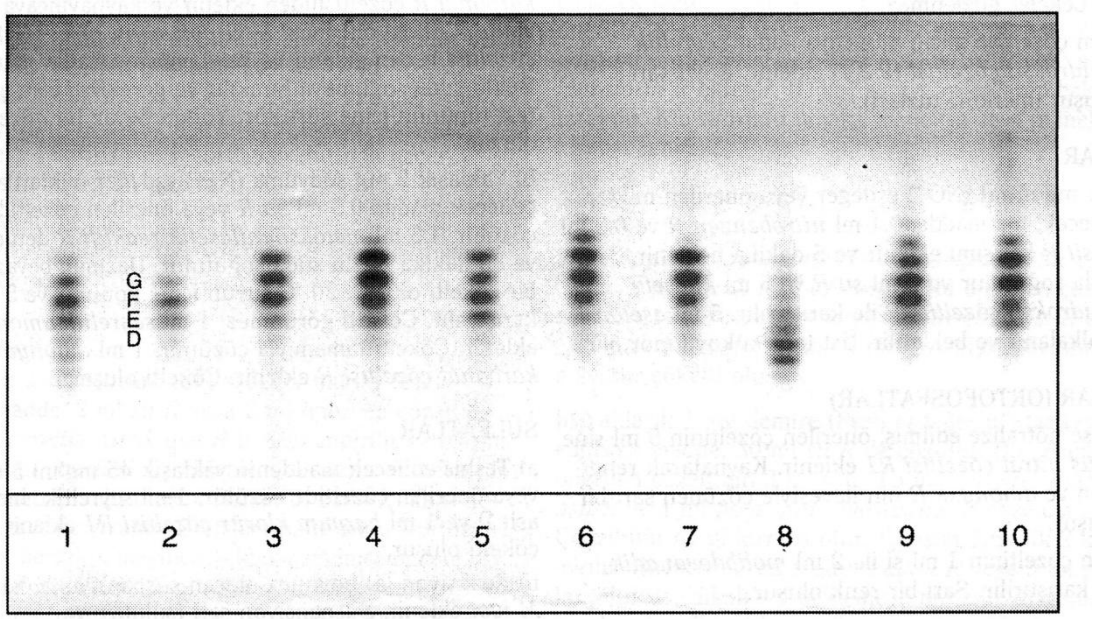
| | |
|---|---|
| Empty flask weight (dead load): A g<br>Flask + oil = B g<br>B – A = C g oil<br>T = Sample amount (g)<br>X = Yield | X = C x 100 / T = yield% |

**Identification of Fatty Oils by Thin-Layer Chromatography**
**EUROPEAN PHARMACOPEIA 6.0,** 2.3.2; 01/2008:20302

Examine by thin-layer chromatography, using as the coating substance a suitable octadecylsilyl silica gel (RP C-18) for high performance thin-layer chromatography.
**Test solutions**. Unless otherwise prescribed, dissolve about 20 mg (1 drop) of the fatty oil in 3 ml of *methylene chlorid R.*
**Reference solution**. Dissolve about 20 mg (1 drop) of maize oil R in 3 ml of of *methylene chlorid R*.

Apply separately to the plate 1 µl of each solution. Develop twice over a path of 0.5 cm using ether *R*. Develop twice over a path of 8 cm using a mixture of 20 volumes of *methylene chloride R*, 40 volumes of *glacial acetic acid R* and 50 volumes of *aceton R*. Allow the plate to dry in air and spray with a 100 g/l solution of phosphomolibdic acid R. Heat the plate at 120 ˚C for about 3 min and examine in daylight.

The chromatogramm obtained typically shows spots comparable to those in Figure 1.




**Chromatograms for identification of fatty oils.**

1. Arachis oil (Yer fıstığı yağı)
2. Olive oil (Zeytin yağı)
3. Sesami oil (Susam yağı)
4. Maize oil (Mısır Yağı)
5. Almond oil (Badem yağı)
6. Soya-bean oil (Soya yağı)
7. Sunflower oil (Ayçiçeği yağı)
8. Rapeseed oil (Kolza yağı)
9. Rapeseed oil, erucic acid-free (Kolza yağı)
10. Wheat-germ oil (Buğday özü yağı)

**IDENTIFICATION OF OIL SAMPLES (EP 6)**

Carry out the test for identification of fatty oils by thin-layer chromatography. The chromatogram obtained shows spots corresponding to those in the typical chromatogram for the related oil.

# PHENOLICS

Phenolics form a vast group of substances which is difficult to define in simple terms. The fundamental structural element that characterizes them is the presence of at least one aromatic ring substituted by at least one hydroxyl group, free or engaged in another function: ether, ester, or glycoside. However, a purely chemical definition of phenols is insufficient to characterize plant phenolics: it would include secondary metabolites which possess these structural elements, but which evidently belong to quite different phytochemical groups.

Only plants and microorganisms are capable of biologically synthesizing the aromatic nucleus. Animal organisms are almost always dependent on either their nutrition or a symbiosis to elaborate indispensable metabolites (There are a few exceptions) comprising this structural element (e.g., amino acids, vitamins, pigments, toxins).

**Plant phenolics arise from two main aromatization pathways**:

- The most common pathway is the one which, via shikimate (shikimic acid), leads from monosaccharides to aromatic amino acids (phenylalanine and tyrosine), then, by deamination of the latter, to cinnamic acids and their numerous derivatives, including **benzoic acids, acetophenones, lignans, lignins, and coumarins**;

- The other pathway begins with acetate and leads to poly-β-ketoesters of variable length—polyketides—which afford, by cyclization (Claisen or aldol condensation), products that are often **polycyclic**, including **chromones, isocoumarins, orcinols, depsides, depsidones, xanthones, and quinones**.

The structural diversity of phenolics is due to this dual biosynthetic origin and is increased by the frequent combination of the ***shikimate and acetate pathways*** in the elaboration of compounds of mixed origin (e.g., **flavonoids** in the broad sense of the term, **stilbenes**, **pyrones**, and **xanthones**). The participation of a third elementary synthon—***mevalonate***—is also possible, although less frequent, and results in mixed derivatives of shikimic acid and mevalonate such as certain quinones or furano- and pyranocoumarins, or in mixed compounds from acetate and mevalonate such as cannabinoids. In a few cases, ail three precursors contribute to the elaboration of one structure, for example that of rotenoids.

Classically, amino acid derivatives that retain the nitrogen atom are considered alkaloids or related substances (e.g., **aromatic amines**, **betalains**).

Due to the great structural diversity of phenolics they are classified in groups designed on the basis of their biosynthetic origin and according to the following outline:

"**shikimates**" (shikimic acid derivatives) and drags containing them;

- **1-phenylpropane derivatives,**
- **1-phenylpropane chain elongation derivatives**,

"**polyketides**" * (*compounds arising chiefly from the cyclization of a poly-β-ketoester*) and drugs containing them.

**"Shikimates"**

**PHENYLPROPANOIDS**

Simple phenols, Cinnamic and Caffeic acid derivatives, Phenylpropanoid glycosides **Coumarins**, Lignans

**"Shikimates"**

**PHENYLPROPANOIDS elongated in Chain**

**DIARYLHEPTANOIDS**, Stilbenoids, Xanthones, Strylpyrones

## DIARYLHEPTANOIDS

### Curcumin

*Reference* : **CLASSIC IN SPECTROSCOPY – Isolation and Structure Elucidation of Natural Producs, Stefan BERGER and Dieter SICKER, WILEY-VCH, Weinheim, 2009**

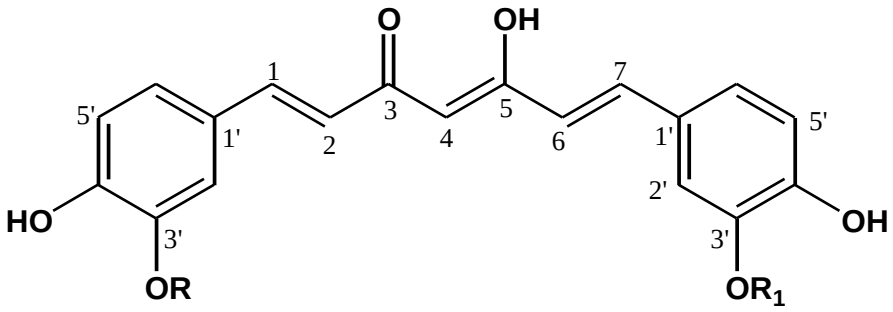
**(1*E*,4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one**

Source: Turmeric, Zerdeçal

*Curcuma longa* (Zingiberaceae)

$C_{21}H_{20}O_6$: MW 368.39

**Curcuminoids**




**Curcumin, R = $R_2$ = $CH_3$**
**Demethoxy-Curcumin, R = H, $R_2$ = $CH_3$**
**Bis-demethoxy-Curcumin, R = $R_2$ = H**

| | |
|---|---|
|  |  |
| ***Curcuma longa***<br><br>**From: Köhler–s Medizinal-Pflanzen** | **Curcuma longa rhizoma** |

*Curcuma longa* (Turmeric, zerdeçal) has been known as an Indian spice. Turmeric is a tropical perennial plant from the ginger family (Zingiberaceae). It is a basic constituent of curry powders and blended with cumin, coriander and fenugreek. Turmeric powder gives a characteristic orange colour due to curcumin and its demethyl derivatives.

Turmeric is also used as "Indian saffron" to give other foods such as mustard a yellow colour because it is much cheaper than real saffron. Turmeric rhizome contains **curcuminoids** at a level of 3-4 %.

Turmeric have been known in India and China for millennia and have been used practically in Ayurvedic and similar systems of traditional medicine. The diseases cured with turmeric include anorexia, biliary disorders, cough, hepatic disorders, diabetic wounds, sinusitis and rheumatism.

In the last decade scientists have recognized the potential of turmeric. This realization initiated a real explosion in research on turmeric ingredients in combination with the desire to find other applications. Curcumin has been found to show, e.g., antioxidant properties, antiinflammatory effects, hypotensive, hypocholesteraemic and anti-cancer properties, haemostatic properties and anti-amyloid properties following oral or topical administration. It has been shown to reduce the severity of cystic fibrosis, Alzheimer's and Parkinson's disease and even stroke. Its mechanisms of action include inhibition of cell signalling pathways and effects on enzymes such as cyclooxygenase and glutathione-S-transferase.
More than 6374 papers have dealt with curcumin, among them over 417 reviews (Web of Science, September 2012).

In summary, turmeric and its main component curcumin have a widespread profile of biological activity.

## 7. TLC Analysis of Curcumin and its Derivatives of Turmeric

**Preparation of Extract**

***Curcuma longa* (Zingiberaceae), Turmeric, Zerdeçal**

A. Powdered drug (1 g) is extracted with methylene chloride (= Dichloromethane, 10 mL) using homogenizator at room temperature for 5 min. The clear filtrate is used for TLC.

**Thin-Layer Chromatography (TLC)**

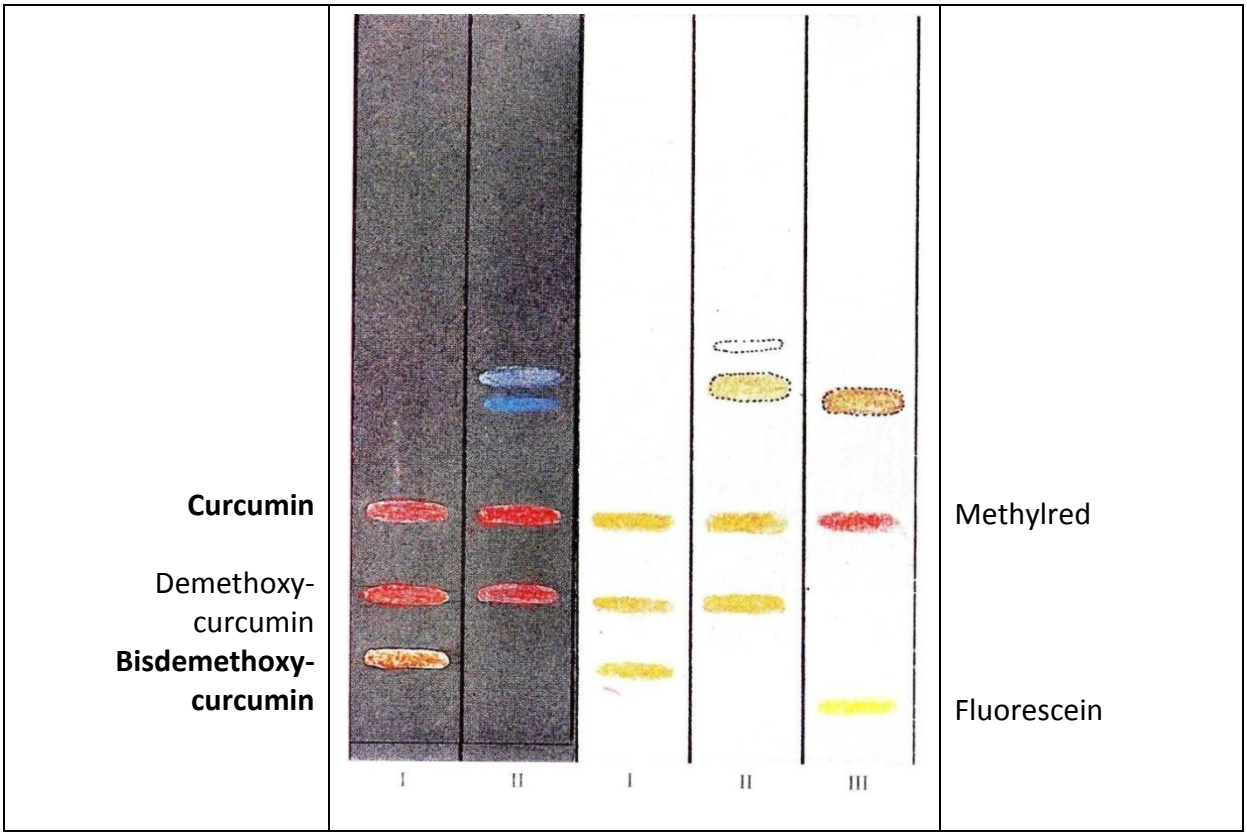
**Reference solution** : A mixture of curcuminoids are dissolved in 1 mL methanol and is used for TLC investigation.
**Adsorbent** : Silica gel 60$F_{254}$ precoated TLC plates
**Solvent system** : Methylene chloride-Methanol (99:1)

**$R_f$ of Curcumin : 0.45**
**$R_f$ of Demethoxycurcumin : 0.20**
**$R_f$ of BisdemethoxyCurcumin : 0.08**

**Detection**

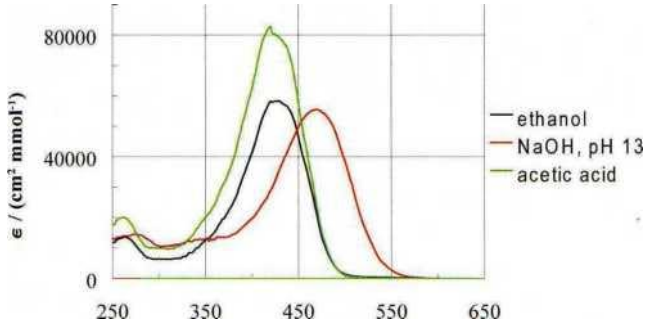
**a.** All curcuminoids show quenching in UV-254 nm.
**b**. All curcuminoids are observed as yellow spots on TLC plate in day light.
**c**. All curcuminoids show pale yellow fluorescence in UV-365 nm.

**TLC Chromatogram**




From: **PHARMAZEUTISCHE BIOLOGIE – 4. Drogenanalyse II: Inhaltsstoffe und Isolierungen**
**Egon STAHL & Werner SCHILD, Gustav Fischer Verlag, Stuttgart, 1981**

**UV Spectra of Curcuminoids***



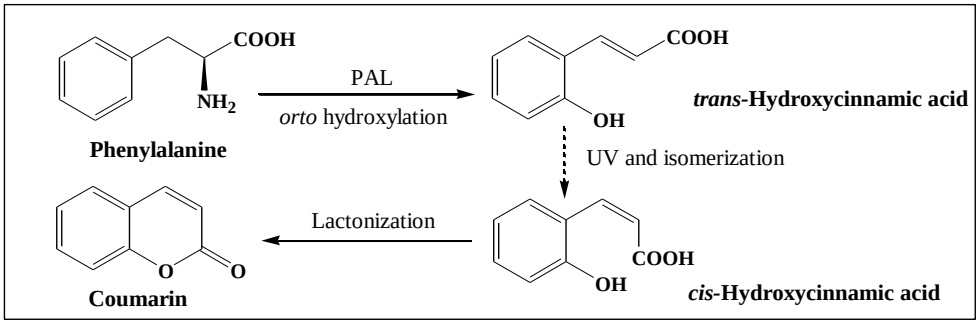

UV spectrum of Curcumin in acetic acid, ethanol and NaOH.

Curcumin has a maximum absorption band at 430 nm.

***UV Spectra of curcuminoids will be recorded using TLC scanner**.

## COUMARINES

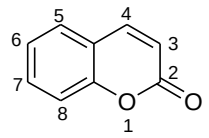
The coumarins are shikimate-derived metabolites formed when phenylalanine is deaminated to *trans*-hydroxycinnamic acid. The double bond of this acid is converted to cis-form by light catalyzed isomerization, resulting lactonization to yield coumarins (benzo-α-pyrone). Coumarin and its derivatives are all considered as phenylpropanoids.



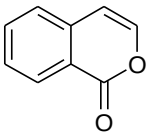

### Simple coumarins

**Coumarins** have clinical medical value as the precursor for several anticoagulants, notably warfarin. Coumarin has blood-thinning, anti-fungicidal and anti-tumor activities. Coumarin should not be taken while using anticoagulants. Coumarin increases the blood flow in the veins and decreases capillary permeability. Coumarin can be toxic when used at high doses for a long period.

**COUMARINS**

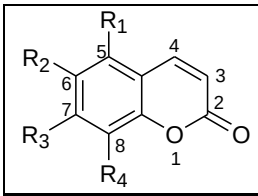



Benzo-α-pyrone
2H-chromen-2-one
**(coumarine)**



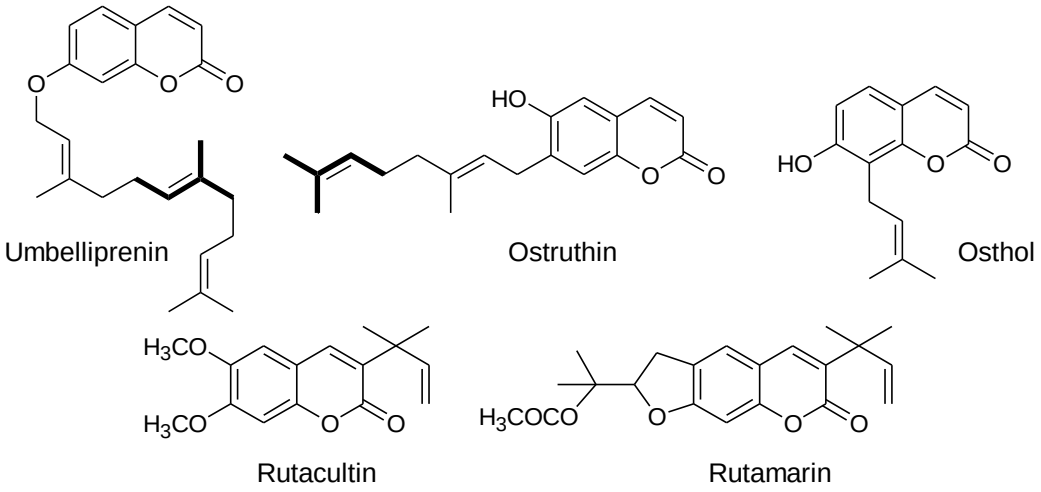

1H-isochromen-1-one
**(isoоumarine)**

**SIMPLE COUMARINS**




| $R_1$ | $R_2$ | $R_3$ | $R_4$ | Coumarins | Chemical Name |
|---|---|---|---|---|---|
| H | H | OH | H | Umbelliferon | 7-hydroxycoumarin |
| H | H | $OCH_3$ | H | Herniarin | 7-methoxycoumarin |
| H | OH | OH | H | Aesculetin | 6,7-dihydroxycoumarin |
| H | H | OH | OH | Daphnetin | 7,8-dihydroxycoumarin |
| H | $OCH_3$ | OH | H | Scopoletin | 6-methoxy-7-hydroxycoumarin |
| H | $OCH_3$ | OH | $OCH_3$ | Isofraxidin | 7-hydroxy-6,8-dimethoxycoumarin |
| $OCH_3$ | H | $OCH_3$ | H | Fraxinol | 6-hydroxy-5,7-dimethoxycoumarin |

**C-PYRENYLATED COUMARINS**



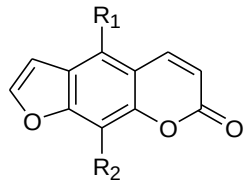

**7,6-FURANOCOUMARINS (Psoralen-type furanocoumarins =** linear-furanocoumarins**)**
**Furanocoumarins** or **furocoumarins**

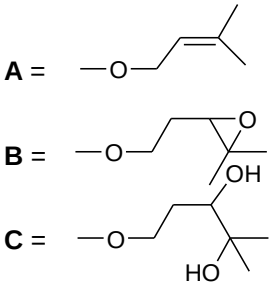
They are biosynthesized partly through the phenylpropanoid pathway and the mevalonate pathway, which is biosynthesized by a coupling of dimethylallyl pyrophosphate (DMAPP) and 7-hydroxycoumarin (umbelliferone).

The chemical structure of furanocoumarins consists of a furan ring fused with coumarin. The furan may be fused in different ways producing several isomers. The two most common isomers are psoralen and angelicin. Derivatives of these two core structures are referred to respectively as linear and angular furanocoumarins.

Many furanocoumarins are toxic and are produced by plants as a defense mechanism against various types of predators ranging from insects to mammals. This class of phytochemical is responsible for the phytophotodermatitis seen in exposure to the juices of the wild parsnip.

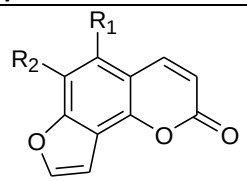
Furanocoumarins have other biological effects as well. For example, in humans, bergamottin and dihydroxybergamottin are responsible for the "grapefruit juic effect", in which these furanocoumarins affect the metabolism of certain pharmaceutical drugs.



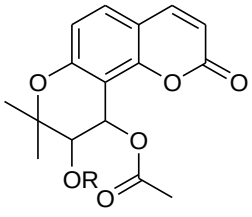

| $R_1$ | $R_2$ | 7,6-furanocoumarines |
|---|---|---|
| H | H | Psoralen |
| $OCH_3$ | H | Bergapten |
| H | $OCH_3$ | Xanthotoxin |
| H | H | Xanthotoxol |
| $OCH_3$ | $OCH_3$ | Isopimpinellin |
| H | **A** | Imperatorin |
| **A** | H | Isoimperotorin |
| **B** | H | Oxypeucedanin |
| **C** | H | Oxypeucadaninhydrat |

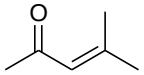
**7,8-FURANOCOUMARINES (Angelisin-type furanocoumarins =** angular-furanocoumarins)

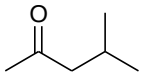


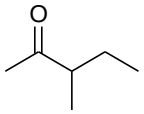
| $R_1$ | $R_2$ | 7,8-furanocoumarines |
|---|---|---|
| H | H | Angelicin |
| $OCH_3$ | H | Isobergapten |
| H | $OCH_3$ | Sphondin |
| $OCH_3$ | $OCH_3$ | Pimpinellin |

**PYRANOCOUMARINS (Visnadin-type)**



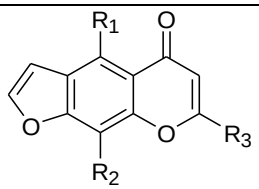
**A** = 

**B** = 

**C** = 

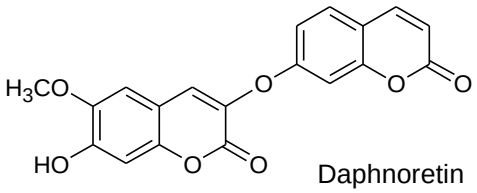
| $R_1$ | pyranocoumarines |
|---|---|
| **A** | Samidin |
| **B** | Dihydrosamidin |
| **C** | Visnadin |

**FURANOCHROMONS**



| $R_1$ | $R_2$ | $R_3$ | furanochromones |
|---|---|---|---|
| $OCH_3$ | $OCH_3$ | $CH_3$ | Khellin |
| OH | $OCH_3$ | $CH_3$ | Khellinol |
| $OCH_3$ | H | $CH_3$ | Visnagin |
| $OCH_3$ | H | $CH_2OH$ | Khellol |
| $OCH_3$ | $OCH_3$ | $CH_2OH$ | Ammiol |

**DIMERIC COUMARINS**



Daphnoretin

## 8. Thin Layer Chromatography (TLC) of coumarin and coumarin derivatives

**Preparation of drug extracts for TLC**:

- Pulver drugs (each 1 g) is extracted in 10 ml MeOH 10 minutes at 50 °C by keeping in ultrasonic bath.

- 0.5 g pulver drug of Ammi visnagae fructus is extracted with 10 ml 60% ethanol 30 min under vibrating. The filtrate is concentrated carefully to approximately 5 ml, of which 20 µl is applied on to the TLC.

TLC

**Reference solution**
**Adsorbent**: TLC Silica gel 60 $F_{254}$

**Mobile phase** (Solvent system): Toluen-Ether (1:1/saturated with acetic acid): In a separating funnel 50 ml toluol and 50 ml Ether are mixed and shaked with 50 ml 10% AcOH several times strongly. The Unterphase is discharged and toluol ether mixture is used for TLC.

**Detection**

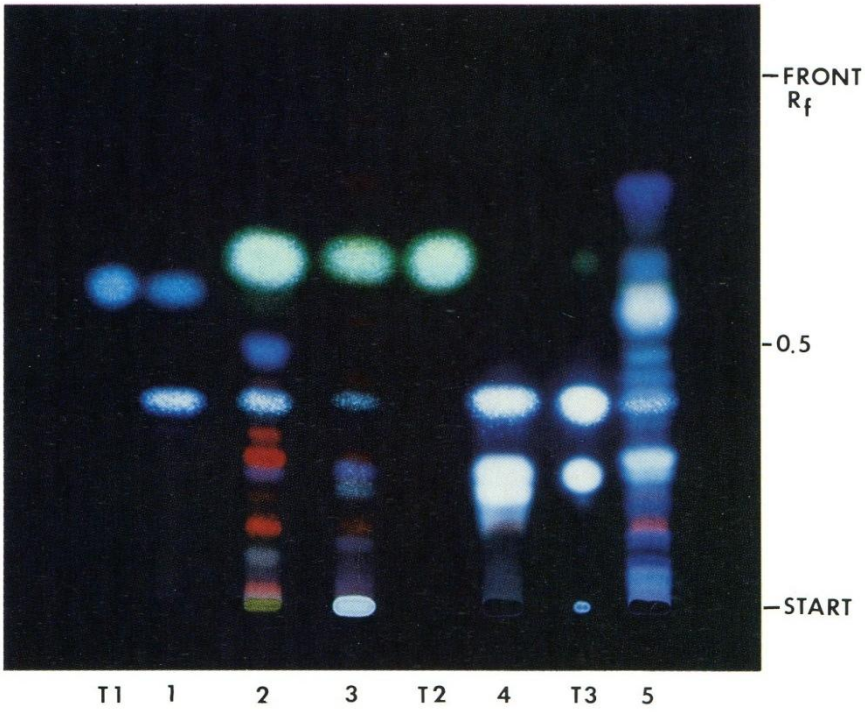
**Direct evaluation**:
$UV_{254}$:
$UV_{365}$:

**Spraying reagents**
Potassium hydroxide (KOH)
Naturstoff-Polyethylenglykol-reagent
Antimon-III-chlorid reagent

A. Drugs containing coumarins

| **Families** | **Drugs** | ***Plants*** | **Main compounds** |
|---|---|---|---|
| **APIACEAE** | Angelicae radix | *Angelica archangelica*<br>*sylvestris* | Coumarines |
| | Imperatoria radix | *Peucedanum ostruthium* | Coumarines |
| | Levistici radix | *Levisticum officinale* | Coumarines, phtalides |
| | Pimpinella radix | *Pimpinella saxifraga*<br>*P. major* | Coumarines |
| | Heraclei radix | *Heracleum spondylium* | Coumarines |
| | Ammi majoris fructus | *Ammi majus* | Coumarines |
| | Ammi visnagae fructus | *Ammi visnaga* | Coumarines |
| | Asa foetida | *Ferula assa-foetida* | Coumarines, ferulic acid esters |
| RUBIACEAE | Asperula herba | *Galium odoratum* | Coumarines |
| FABACEAE | Meliloti herba | *Melilotus officinalis* | Coumarines |
| CARYOPHYLLACEAE | Herniariae herba | *Herniaria glabra*<br>*H. hirsuta* | Coumarines, flavonoids saponines |
| RUTACEAE | Rutae herba | *Ruta graveolens* | |
| ASTERACEAE | Abrotani herba | *Artemisia abrotanum* | Coumarines |
| OLEACEAE | Fraxini cortex | *Fraxinus excelsior*<br>*F. ornus* | Coumarines |
| THYMELACEAE | Mezerei cortex | *Daphne mezereum* | Coumarines |
| SOLANACEAE | Scopoliae radix | *Scopolia carniolica* | Coumarines, alkaloids |

**TLC of Coumarin Drugs (Herniariae-, Meliloti-, Asperulae-, Abrotani- and Rutae herba)** [1/2]




**Chromatogram A**

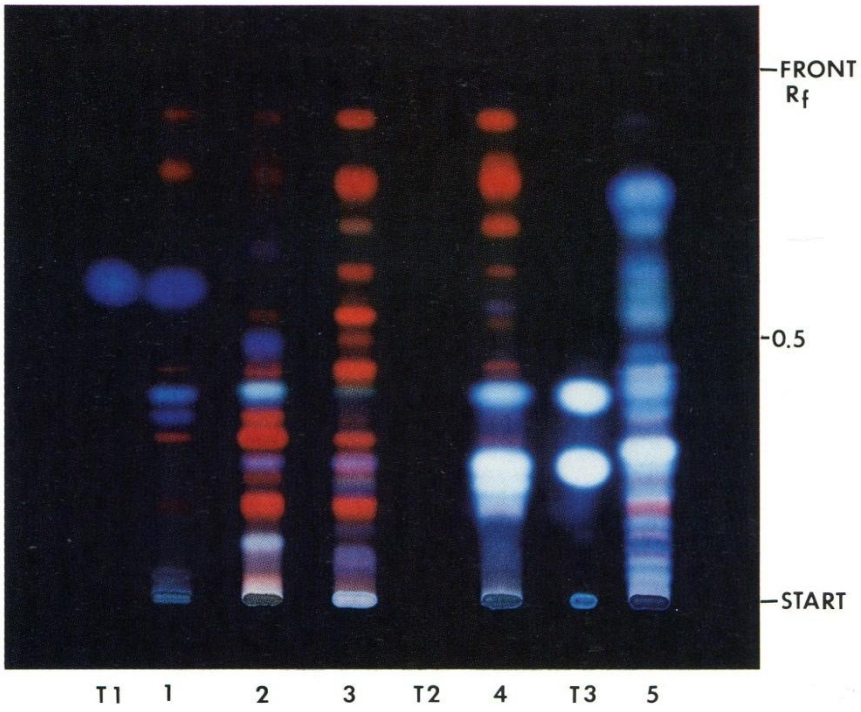
**Herbal Drugs**

**1. Herniariae herba**
**2. Meliloti herba**
**3. Asperulae herba**
**4. Abrotani herba**
**5. Rutae herba**

**Test solutions**

T1. Herniarin
T2. Coumarin
T3. Scopoletin

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Toluol – Ether (1:1 / saturated with AcOH)
**Detection**: Direct observation under UV-365 nm (**Chromatogram A**).




Chromatogram B

**Herbal Drugs**

1. Herniariae herba

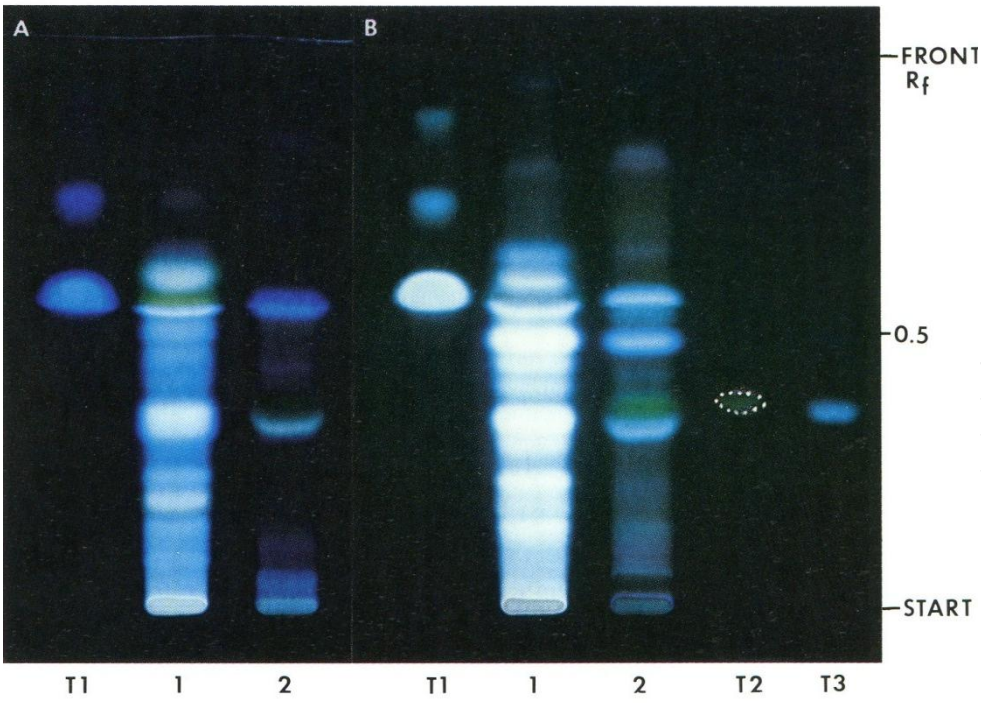
Test solutions
T1. Herniarin
T2. Coumarin
T3. Scopoletin

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Toluol – Ether (1:1 / saturated with AcOH)
**Detection**: KOH reagent* and observation under UV-365 nm (**Chromatogram B**).
**KOH R.**: 5% KOH solution in EtOH. It is sprayed to TLC plate and evaluated under UV-365 nm without heating.

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen, Springer-Verlag, Berlin, 1983.

## TLC of Coumarin Drugs (Ammi fructus)



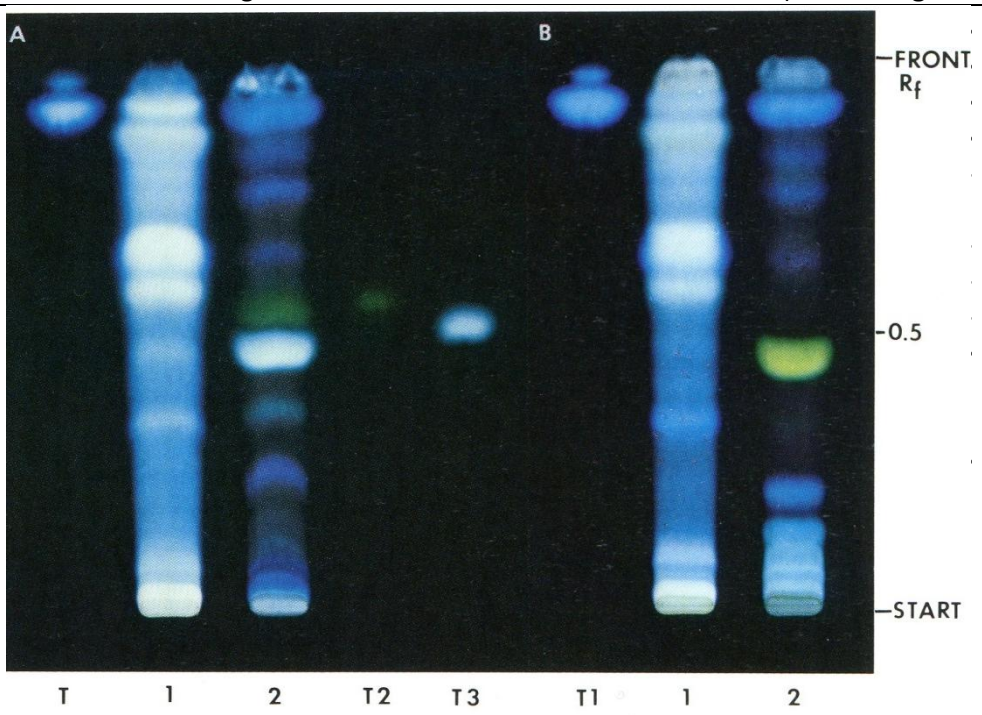



**Chromatograms A & B**

**Herbal Drugs**
1. Ammi fructus (*Ammi major*)
2. Ammi fructus (*Ammi majus*)

**Test solutions**
T1. Visnadin
T2. Khellin
T3. Visnagin

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase - 1:** Toluol – Ether (1:1 / saturated with AcOH)
**Detection**: Direct observation under UV-365 nm (**Chromatogram A**).
KOH reagent and then observation under UV-365 nm (**Chromatogram B**).




**Chromatograms A&B**

**Herbal Drugs**
1. Ammi fructus (*Ammi major*)
2. Ammi fructus (*Ammi majus*)

**Test solutions**
T1. Visnadin
T2. Khellin
T3. Visnagin

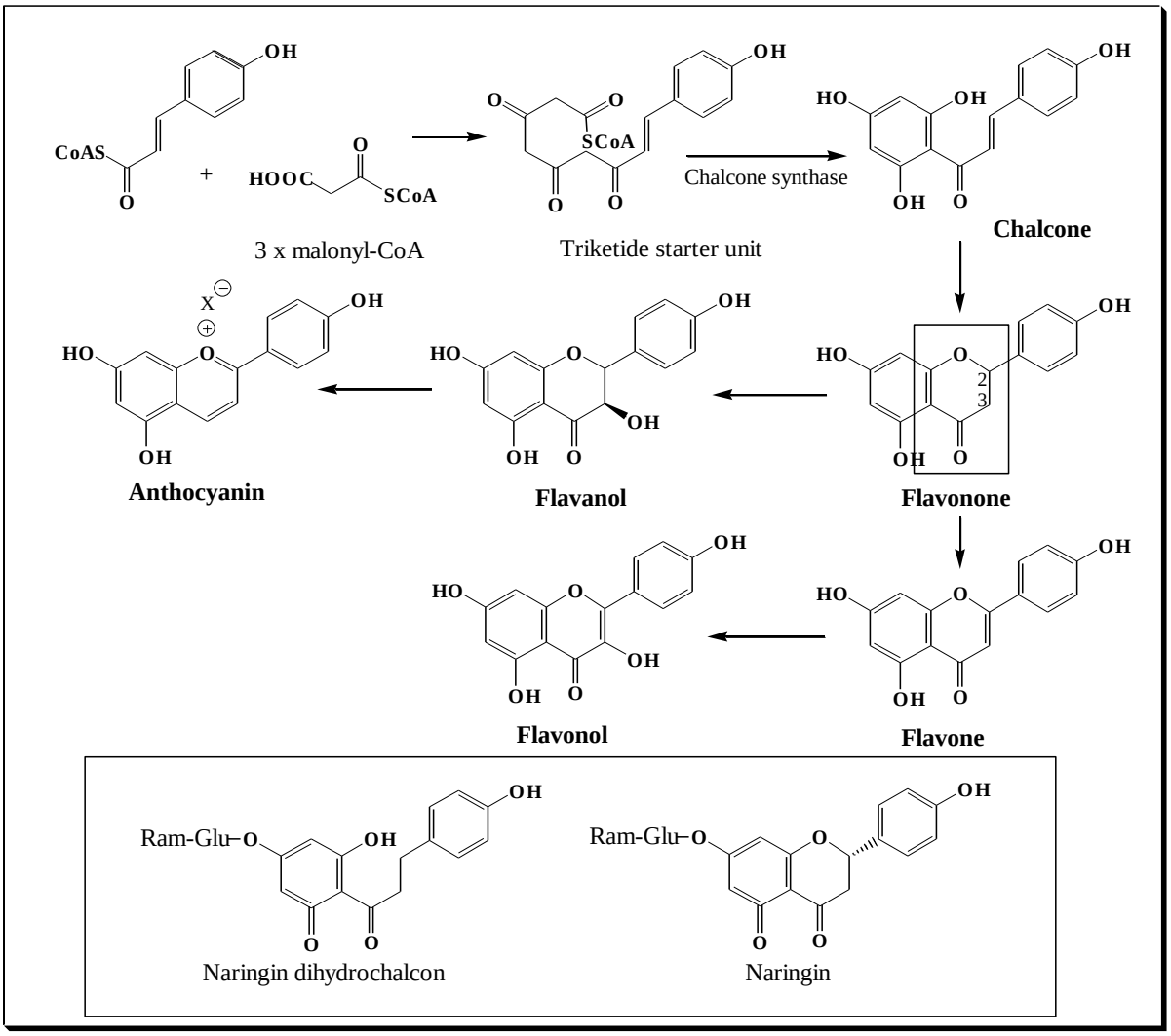
**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate (EtOAc)
**Detection**: KOH Reagent and observation under UV365 nm (**Chromatogram A**).
$SBCl_3$-Reagent*and observation under UV365 nm (**Chromatogram B**).
**$SBCl_3$-reactive**: 20% Antimon-III*chlorid solution in chloroform.

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen, Springer-Verlag, Berlin, 1983

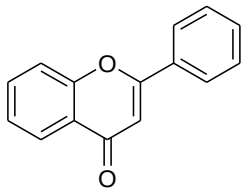
## FLAVONOIDS

The flavonoids are derived from a $C_6$-$C_3$ (phenylpropane) unit which has its source shikimic acid (via phenylalanine) and a $C_6$ unit that is derived from the polyketid pathway. This polyketide fragment is generated by three molecules of malonyl-CoA, which combine with the $C_6$-$C_3$ unit to form a triketide starter unit. Flavonoids are therefore of mixed biosynthesis, consisting of units derived from both shikimic acid and polyketide pathways.

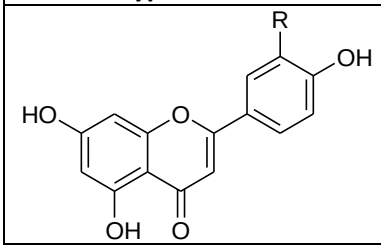
The triketide starter unit undergoes cyclization by the enzyme *chalcone synthase* to generate the **chalcone** group of flavonoids. Cyclization can then occur to give a pyranone ring containing **flavonone** nucleus, which can either have the C(2)-C(3) bond oxidized (unsaturated) to give the **flavones** or be hydroxylated at position C(3) of the pyranone ring to give the **flavanol** group. The **flavanols** may then be further oxidized to yield the **anthocyanins**. The flavones can be hydroxylated at C(3) position to give **flavonols**.

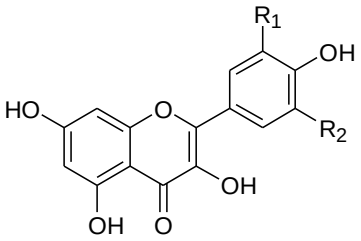


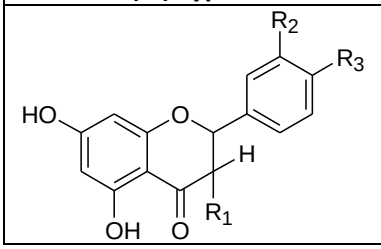

The flavonoids contribute to many other colours found in nature, particularly the yellow and orange of petals. These compounds have high ecological importance in nature as colour attractants to insects and birds as an aid to plant pollination. Certain flavonoids also markedly affect the taste of foods. The flavonone glycoside naringin, which occurs in the peel of grapefruit, is very bitter and astringent. However, naringin dihydrochalcone is exceptionally sweet, being some 1000 times sweeter than sucrose. Flavonoids have important dietary significance, being phenolic compounds, they are strongly antioxidant.

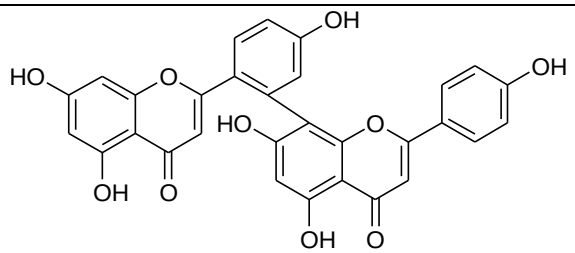


**Flavone**
2-phenyl-1,4-benzopyrone

| **Flavone-type** | **R** | **Aglycone** | **Glycoside** |
|---|---|---|---|
|  | H | Apigenin | Apigenin 8-C-glucoside (= Vitexin)<br>Vitexin 2''-*O*-rhamnoside |
| | OH | Luteolin | Luteolin 8-C-glucoside (=Orientin) |

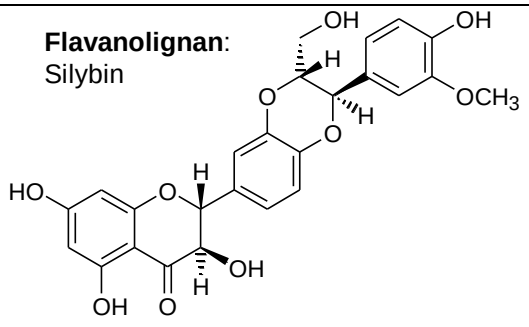
| **Flavonol-type** | **$R_1$** | **$R_2$** | **Aglycone** | **Glycoside** |
|---|---|---|---|---|
|  | OH | H | Quercetin | Q 3-*O*-galactoside (= Hyperoside)<br>Q 3-*O*-glucoside (= Isoquercitrin)<br>Q 3-*O*-rhamnoside (= Quercitrin)<br>Q 3-*O*-rutinoside (= Rutin) |
| | H | H | Kaempferol | K 3-*O*-glucoside (= Astragalin) |
| | OH | OH | Myrcetin | M 3-*O*-digalactoside (= Hyperoside) |
| | $OCH_3$ | H | Iso-rhamnetin | I 3-*O*-rutinoside (= Narcissin) |

| **Flavonon(ol)-type** | **$R_1$** | **$R_2$** | **$R_3$** | **Aglycone** |
|---|---|---|---|---|
|  | H | H | OH | Naringenin |
| | H | OH | OH | Eriodictyol |
| | H | $OCH_3$ | OH | Homoeriodictyol |
| | H | OH | $OCH_3$ | Hesperetin |
| | OH | H | OH | Taxifolin |



**Biflavone**: Amentoflavon

**Flavanolignan**: Silybin

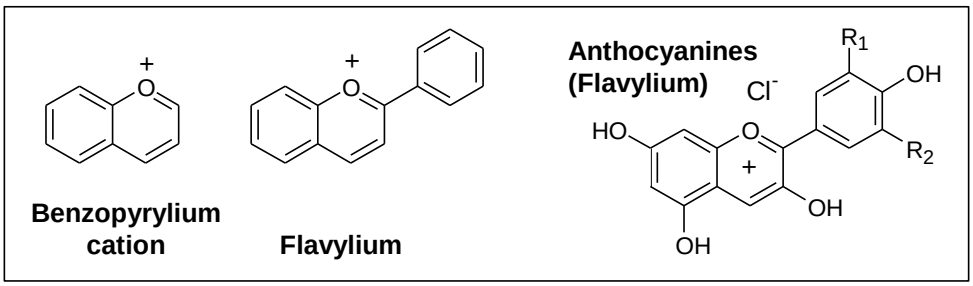


## Flavonoid containing Herbal Drugs

| **Families** | **Drugs** | ***Plants*** | **Main flavonoids** |
|---|---|---|---|
| Asteraceae | Arnicae flos | *Arnica montana* | Quercetin glycosides |
| | Anthemidis flos | *Anthemis nobilis (syn.: Chamaemelum nobile)* | Apigenin and luteolin glycosides |
| | Calendula flos | *Calendula officinalis* | Isorhamnetin and Quercetin glycosides |
| | Farfarae flos | *Tussilago farfara* | Quercetin glycosides |
| | Matricaria flos | *Chamomilla recutita* | Quercimeritrin, apigenin, luteolin, patuletin glycs. |
| | Cardui mariae fructus | *Silybum marianum* | **Flavonolignans**: Silybin, silychristin, silydianin |
| Primulaceae | Primula flos | *Primula veris, P. elatior* | Quercetin and Gossypetin glycosides |
| Rosaceae | Crataegi flos | *Crataegus monogyna C. pentagyna, C. nigra, C. azarolus* | Quercetin, apigenin glycosides, flavon C-glycosides (vitexin) |
| Caprifoliaceae | Sambuci flos | *Sambucus nigra* | Quercetin glycosides |
| Tiliaceae | Tilia flos | *Tilia cordata* | Quercetin, kaempferol and myrcetin glycosides |
| Scrophulariaceae | Verbasci flos | *Verbascum phlomoides V. thapsiforma* | Flavonol-glycosides Rutin, hesperidin |
| Betulaceae | Betulae folium | *Betula pendula B. pubescens* | Quercetin and myrcetin glycosides |
| Juglandaceae | Juglandis folium | *Juglans regia* | Hyperoside |
| Rutaceae | Aurantii pericarpium | *Citrus aurantium* ssp. *aurantium* | Eriocitrin, rutin Naringenin, naringin, hesperidin, neohesperidin, sinensetin |
| Rutaceae | Citri pericarpium | *Citrus medis* | Eriocitrin, rutin, Neohesperidin, naringenin 7-*O*-hesperidoside |
| Lamiaceae | Orthosiphonus folium | *Orthosiphon spicatus* | **Flavonoid aglycones**: Sinensetin (pentamethoxyflavon), scutellareintetramethylether, eupatorin, |

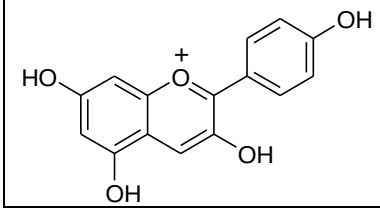
## ANTHOCYANINS AND BETALAINS

Anthocyanins are the substances responsible for the color of the flowers (from the greek *anthos*, flower and kuanos, *blue*). This term is applied to a group of water-soluble pigments responsible for the red, pink, mauwe, purple, blue, or violet color of most flowers and fruits.
These pigments occur as glycosides (the anthocyanins), and their aglycones (the anthocyanidins) are derived from the 2-phenylbenzopyrylium cation.



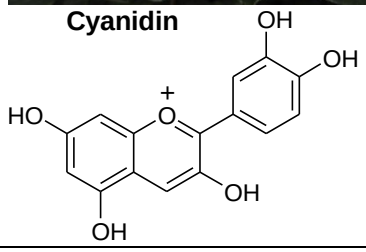

*Pelargonium* spec.

**Pelargonidin**



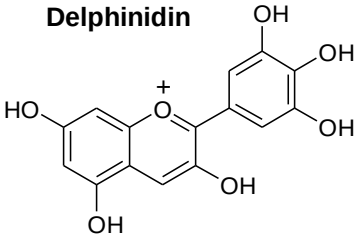
*Rosa* spec.

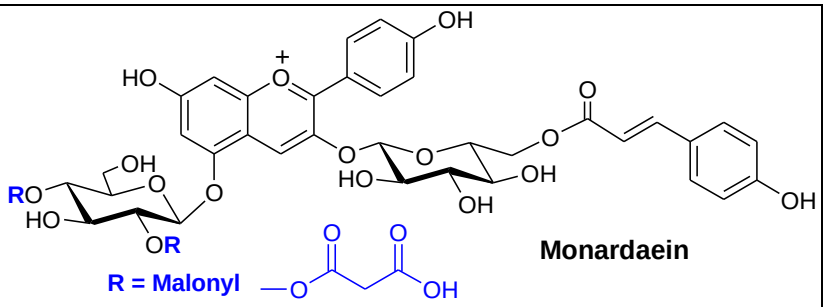
**Cyanidin**



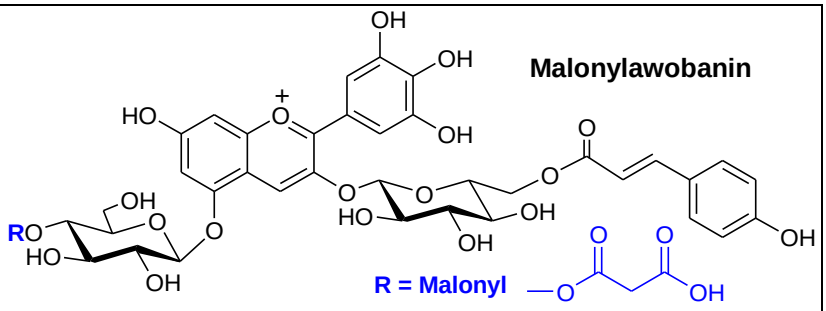
*Delphinium* spec.

**Delphinidin**






*Monarda didyma*




Anthocyanins are present in all of the Angiosperms, except for most taxon of Centrospermae (Caryophyllales, Chenopodiales). In this Order, only the Caryophyllaceae and the Molluginaceae members contain anthocyanins. In other families in the Caryophyllales, the pigmentatition of the various organs is due to betalains (examples: Beet root and bougenvillea or amaranth flowers.

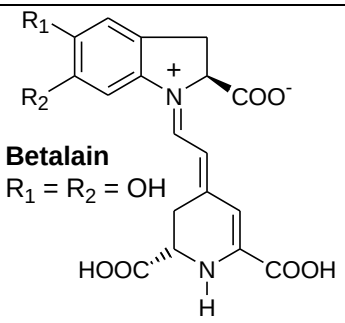

*Carpobrotus edulis* (Aizoaceae)

**Centrospermae (=Caryophyllales, Chenopodiales)**

**Families**:
Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, **Caryophyllaceae**, Chenopodiaceae, Didiereaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae, **Molluginaceae**


*Malephora crocea* (Aizoaceae)

The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red color of beets, bougainvillea, amaranth, and many cacti results from the presence of betalain pigments.




$R_1 = R_2 = OH$, **Betanidin**
$R_1$ = Glucose; $R_2 = OH$, **Betanin**


*Bougainvillea* spec. (Nyctaginaceae)

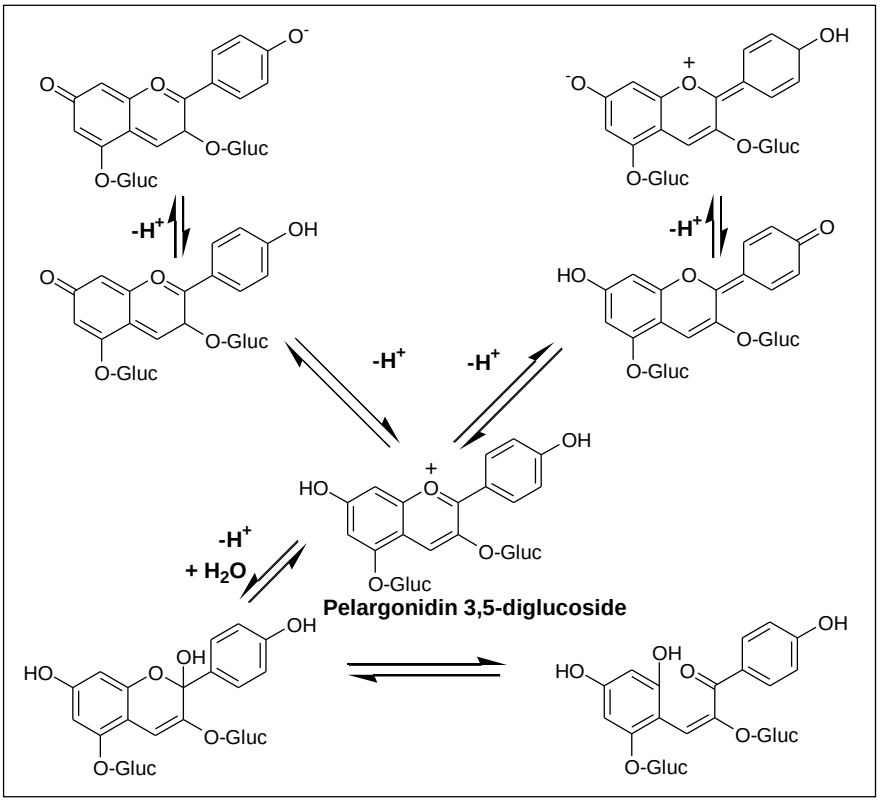

*Amaranthus tricolor* (Amaranthaceae)

There are two categories of betalains: i) **Betacyanins** include the reddish to violett betalain pigments. ii) **Betaxanthins** are those betalain pigments which appear yellow to orange.

**Physico-chemical Properties**

The behavior of anthocyanins in aqueous solution results from the properties of the 2-phenylbenzopyrylium cation, which is at the same time a weak diacid and a good electrophilic reagent. In strong acidic medium (pH<3), the cationic form is red and stable. In weak acidic medium

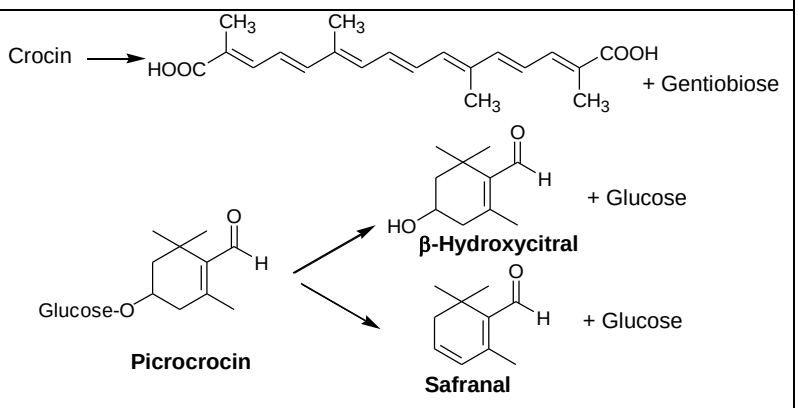
(at pH 4 – 6), the cation loses one or two protons, and this leads to an anhydrobase, which is neutral or ionized, respectively, and stabilized by resonance; these quinonoid forms are blue. Hydrating the molecule (in the 2-position, by simple dilution) leads to a carbinol pseudobase, which is colorless, and if pH increases, it becomes ionized (as a phenate), and the anthocyanin structure is destroyed. As consequence of these properties, neutral and slightly acidic anthocyanin solutions lose their color fairly rapidly (although the anhydrobase is colored, the hydration of the cation is faster than the loss of a hydroxyl hydrogen). To explain the color of anthocyanins at the usual pHs of living media – since the pH of vacuoles is only very rarely lower than 3 -, it is necessary to take into account the stabilization of their structure and its protection against the nucleophilic addition of water. Several mechanisms may be at play: intramolecular copigmentation, in other words acylation by cinnamic acids (acylated anthocyanins are remarkably stable in solution), intermolecular pigmentation with flavonoids, metal chelation (to form metalloanthocyanins), and self association, also referred to as auotocopigmentation.



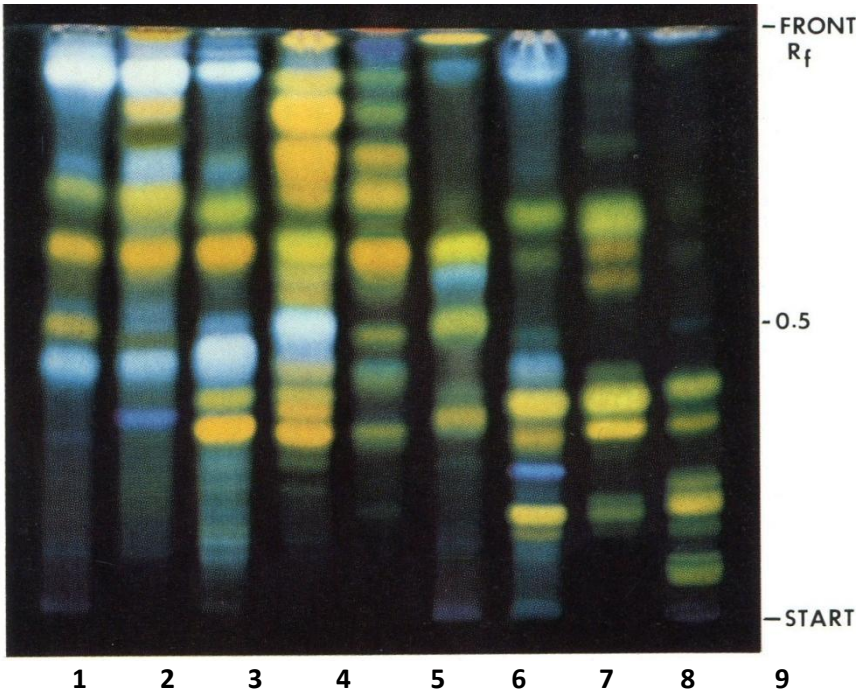

Structural transformation of anthocyanins in aqueous solution.

***Crocus* Pigments**

*Crocus sativus* (Safran)



## 7. TLC of Flavonoid Drugs (Flowers – flos-Pericarps)

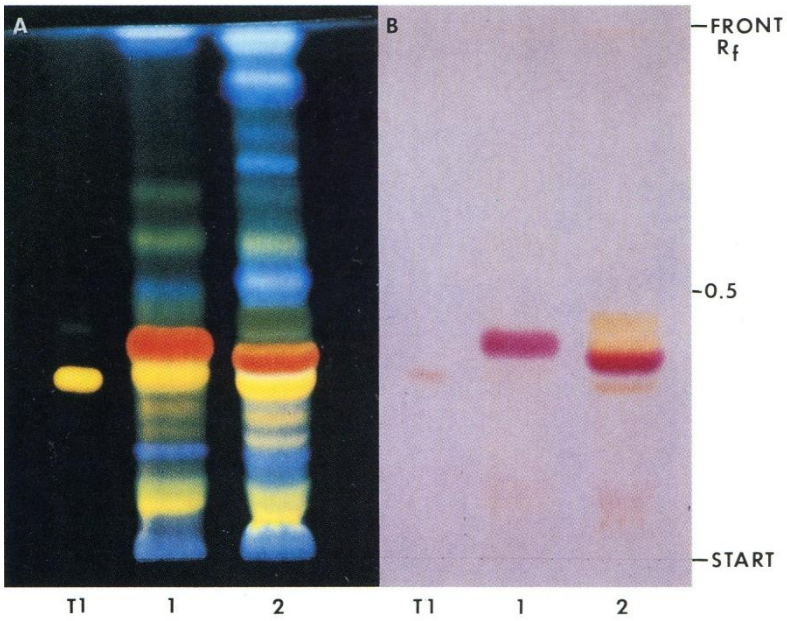



**Chromatogram A**

**Herbal Drugs**
**1. Arnicae flos**
**2. Stoechados flos**
**3. Sambuci flos**
**4. Pruni spinosa flos**
**5. Tiliae flos**
**6. Verbasci flos**
**7. Calendulae flos**
**8. Cacti flos**
**9. Primulae flos**

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate – Formic acid – Acetic acid – Water (100:11:11:27)
**Detection**: Spray Naturstoff R.* (**Chromatogram A**) on the plate, observe under UV-365 nm. .
***Natustoff R.:** 1% Diphenylboryloxyethylamin solution in MeOH

**A** **B**




**Chromatograms B&C**

**Herbal Drugs**
**1. Citri pericarpium**
**2. Aurantii pericarpium**

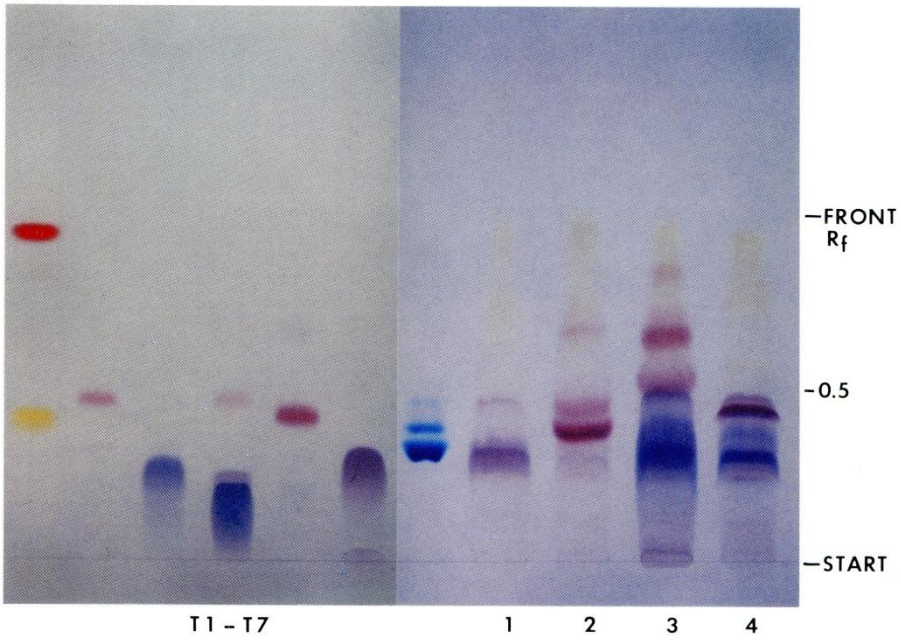
**Test solutions**
T1. Rutin
T2. Homoeriodictyol

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate – Formic acid – Acetic acid – Water (100:11:11:27)
**Detection**:
**Chromatogram B**: Spray Naturstoff-Polyethylenglycol R. on the plate, observe under UV-365 nm.
**Chromatogram C**: Spray Naturstoff-Polyethylenglycol R. on the plate, observe under day light.

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen, Springer-Verlag, Berlin, 1983.

## 9b. TLC of Anthocyanin and other pigment Drugs

**Chromatogram A** **Chromatogram B**

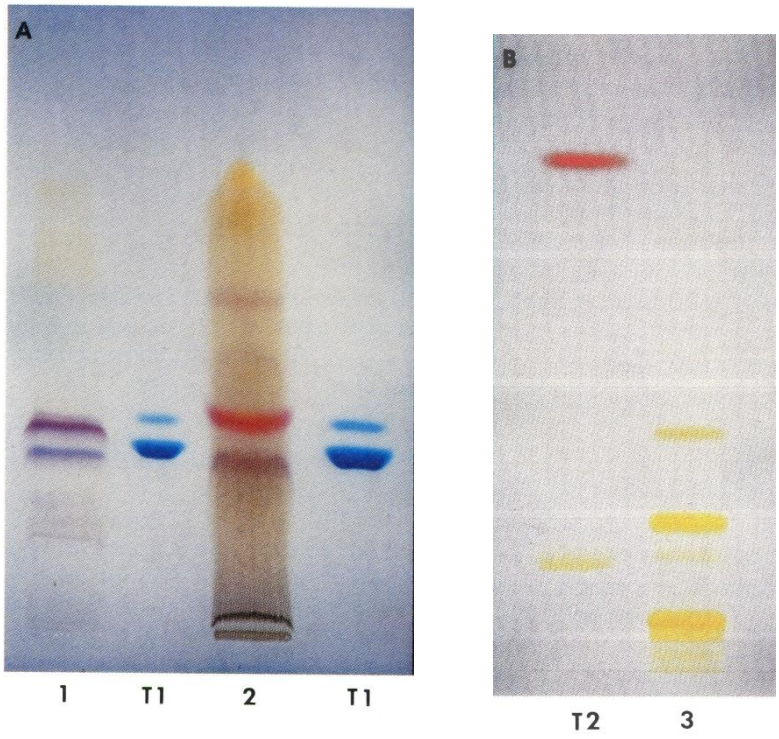



Chromatogram A
**Test solutions**
T1. Naphtolyellow + Sudan red
T2. Paeonidin glucoside
T3. Petunidin 3,5-digluc
T4. Delphinidin di- & monoglucosides
T5. Malvidin monogluc
T6. Cyanidin 3,5-digluc
T7. Methylenblue

Chromatogram B
**1. Cyani flos**
**2. Malva sylvestris flos**
**3. Malva arboreae flos**
**4. Hibisci flos**

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** n-BuOH– Acetic acid – Water (40:10:20)
**Detection**: Direct evaluation under the day light.

**A** **B**




Chromatograms A&B
**1. Hibisci flos**
**2. Paeoniae flos**
**3. Croci stigma**

Test solutions T1. Methylen blue
T2. Naphtol yellow

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:**
n-BuOH– Acetic acid – Water (40:10:20) (**For chromatogram B**)
Ethylacetate – Isopropanol – Water (65:25:10) (**For chromatogram A**)
**Detection**: Direct evaluation under the day light (**For chromatograms A&B**).

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen, Springer-Verlag, Berlin, 1983.

## 9a Thin Layer Chromatography (TLC) of flavonoid drugs

**Preparation of drug extracts for TLC**:
Drugs: One of the drugs listed above.

Pulver drugs (each 1 g) is extracted in 10 ml MeOH at 60° C for 5 minutes by keeping in ultrasonic bath. The filtrate is concentrated to a few ml, of which 20 µl is applied on to the TLC.

**Exceptions**:

**Cardui mariae fructus** (**Slybi fructus**): 1 g Pulver drug ist extracted first with 50 ml petroleum ether under reflux to remove lipofilic compounds. The defatted drug is further extracted with 10 ml MeOH for 10 min. The filtrate is concentrated to 5 ml, of which 30 µl is applied on to the TLC.

**Farfara folium, Petasites folium**: Pulver drug (each 2 g) is extracted with 10 ml dichloromethan (DCM) for 15 min under reflux. The filtrate is concentrated to a few ml, of which 20 µl is applied on to the TLC.

**Reference solution:** Each of the available reference compound is dissolved in MeOH (0.05%). At least 10 µl of the reference solution should be applied on TLC plate.

**Adsorbent**: TLC Silica gel 60 $F_{254}$

**Mobile phase** (Solvent system):

1. Ethylacetate – Formic acid – Acetic acid – Water (100:11:11:27)
2. Ethylacetate – Acetic acid – Water (66:15:20)
3. Ethylacetate – Formic acid – Acetic acid – Ethylmethylketon- Water (50:7:3:30:10)
4. Chloroform – Aceton – Formic acid (75:16.5:8.5)
5. Chloroform – Ethylacetate (60:40)
6. n-BuOH – Acetic acid – Water (40:10:50; upper phase) (***For cellulose plates***)

**Detection:**

**1. Direct evaluation**:

$UV_{254}$: All flavonoides give a fluorescence reduction in form of dark-blue zones on yellow background of the plates..

$UV_{365}$: According to their structure types, flavonoids represent yellow, blue or green fluorescence.

**Spraying reagents**

i. **Modified Naturstoff –Reagent**: 1% Diphenylboryloxyethylamin solution in MeOH

After spraying, immediately and/or after 15 min, typical intensive fluorescence colors in the UV 365 nm are developed. The additive of PEG (Polyethylene glycol) leads also to an increase of the detection limit (from 10 µg to 0,5 µg). The fluorescence behavior is structural-dependent.

***Flavonols***: Quercetin and myrcetin glycosides give orange

***Kaemferol*** and isorhamnetin glycosides give yellowish green

***Flavons***: Luteolin glycosides give orange colours while apigenin glycosides yellowish green.

## Distinction Between Anthocyanins and Betacyanins

Anthocyanins are almost universal in their occurrence in plants. However, they are replaced by a different type of water-soluble purple pigment in the **Centrospermae**. Plants in all but one of the families of this order have **nitrogen-containing betacyanins**. The most common betacyanin is **betanin**, the pigment of the beetroot. Other members of the Centrospermae from which betacyanins have been isolated are various ***Cactus*** (cacti), ***Bougainvillea*** (Cemile), ***Phytolacca*** (pokeweed, şekerciboyası) and ***Amaranthus*** *(amaranth, horozibiği).*

Betacyanins differ from anthocyanins in being much more unstable to acid hydrolysis, in their colours at different pHs and in their chromatographic and electrophoretic properties, so that it is easy to distinguish them by simple colour tests. Since the two classes of pigment are mutually exclusive in their occurrence *(i.e.* they never occur together in the same plant), these tests can be carried out on crude pigment extracts. Betacyanins also differ from anthocyanins in that, when ingested in food *(e.g.* as **beetroot**, şeker pancarı) they are not metabolized by some 14% of the human population and their colour is excreted unchanged in the urine; hence the condition known as '**beeturia'**.

***Procedure***

(1) Methanolic HCl extracts can be prepared from any *one* of the many anthocyanin sources *(e.g.* any red to purple flowering plant), solution (A) and from beetroot, solution (B) as being the most accessible beta- cyanin source. After maceration, the extracts should be filtered (with celite) and concentrated *in vacuo* to about one fifth of the original volume.

(2) Tests, as indicated in Table , are carried out on both extracts (A-B). The results obtained with A and B are then used to decide what pigments are present in (for unknown samples). The first four tests shown in Table are sufficient for most purposes; the latter two tests can be added if the necessary time and equipment are available.

### *Test for Distinguishing Anthocyanin and Betacyanin*

| *Test* | *Anthocyanin response* | *Betacyanin response* |
|---|---|---|
| *Heat with 2**M** HC1 for 5 min at 100° | colour stable (can be extracted into amyl alcohol) | colour vanishes |
| *Add 2**M** NaOH dropwise | changes to blue-green and slowly fades | changes to yellow |
| *Chromatography in 1 % aqueous HCl | low to intermediate ***Rf*** | high ***Rf*** |
| *Chromatography in BAW | moderate ***Rf*** (10-40) | very low ***Rf*** (00-10) |
| Visible spectrum in Methanol-HCl | maximum in the range 505 to 535 nm | maximum in the range 532 to 554 nm |
| Paper electrophoresis at pH 2-4 | moves towards cathode | moves towards anode |

*The colour tests can be carried out on crude pigment extracts; for anthocyanin, any deep red coloured flowers may be extracted or certain fruits *(e.g.* ***Rubus caesius***, blackberry, böğürtlen; ***Rubus ideaus*** , raspberry,ahududu; ***Fragaria vesca***, strawberry, çilek); for betacyanin, ***Beta vulgaris*** (beetroot, şeker pancarı) extract is convenient. Ref.: **Phytochemical Methods**, J.B. Harborne, Chapman And Hall, London, 1973.

### *Additional Experiments*

(1) Anthocyanins in coloured petals in any series of ornamental plants available may be separated by PC (**P**aper **C**hromatography) in **BAW** (BuOH – **A**cOH – **W**ater) and 1% HCl of methanolic HCl extracts of fresh tissues. Marker solutions of pelargonidin 3,5- diglucoside (from ***Pelargonium*** flowers, sardunya), cyanidin 3,5-diglucoside (most ***Rosa***, red roses, kırmızı gül) and delphinidin 3,5-diglucoside *(**Verbena**, yer minesi; **Delphinium**, hezaren)* should be included on all chromatograms. The nature of the aglycone and the approximate number of sugar residues can be determined from $R_f$*s* and visible colours for each of the flower anthocyanins which separate.

(2) Anthocyanins in different colour types of the same plant may be compared by PC in BAW.

## 9b. Thin Layer Chromatography (TLC) of Anthocyanins and Crocin

Anthocyanin and Crocin containing Herbal Drugs

| **Families** | **Drugs** | ***Plants*** | **Main flavonoids** |
|---|---|---|---|
| Asteraceae | Cyani flos | *Centaurea cyanus* | Cyanin and pelargonidin glycosides |
| Malvaceae | Hibisci flos | *Hibiscus sabdariffa* | Hibiscin (Delphinidin glycoside) |
| | Malvae flos | *Malva sylvestris* | Malvin (Malvidin diglucoside) |
| | Malvae (arboreae) flos | *Althaea rosea* | Delphinidin and malvidin glucosides |
| Paeoniaceae | | | |
| **Crocin and adulteration (False saffron)** | | | |
| Iridaceae | Croci stigma | *Crocus sativa* | Crocin, picrocrocin |
| Asteraceae | Carthamus flos | *Carthamus tinctoria* | |

**Extraction and characterization**

Anthocyanins are soluble in water and alcohols, and insoluble in apolar organic solvents. They are generally extracted with an alcohol (MeOH, EtOH) in the presence of a small amount (0.1 – 1%) of hydrochloric acid. To avoid esterification of the free carboxyl group of acylated anthocyanins by a diacid, and especially to prevent their deacylation, it is beter to use other acids (acetic acid, tartaric acid), and to work at low temperature. Anthocyanin solutions are very unstable, and they can only be kept under nitrogen, at low temperature, and in the dark.

**Preparation of drug extracts for TLC**:

Drugs are listed above.

**Anthocyanin drugs**: Pulver drugs (each 1 g) is extracted with 6 ml a mixture of MeOH and 25% HCl (9:1) for 10 minutes by keeping in ultrasonic bath. After filtration, 25 µl is applied on to the TLC.

**Croci stigma**: 4 – 5 dried Crocus sativus stigma are moistened with water: After 3 min 1 ml MeOH is added and extracted by shaking for 10 min in dark. It can be directly applied on to TLC plate.

**Reference solution:**

***Anthocyanins*** (each 1 mg) is dissolved in 1 ml MeOH.. At least 5 µl is applied on TLC plate.
***Methylene blue***: 5 mg is dissolved in 10 ml MeOH.
***Naphtol yellow***: 5 mg is dissolved in 5 ml MeOH.
***Sudan red***: 5 mg is dissolved in 10 ml MeOH

**Adsorbent**:

TLC Silica gel 60 $F_{254}$
TLC Cellulose

**Mobile phase** (Solvent system):

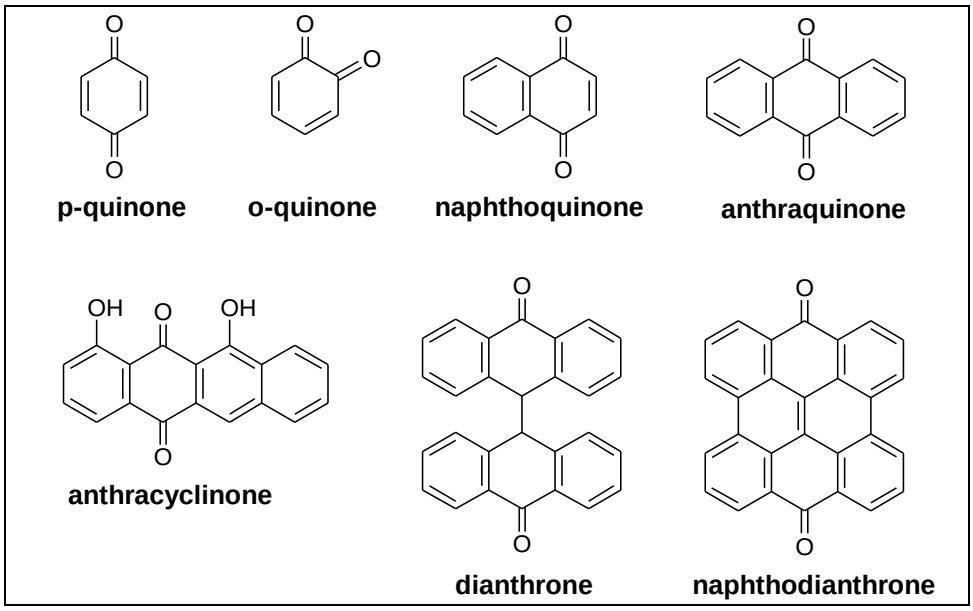
***Anthocyanins***: n-BuOH – Acetic acid – Water (40:10:20) (***For cellulose and Silica gel plates***)
Croci stigma: Ethylacetate – isopropanol – Water (65:25:10)

**Detection:**

**1. Direct evaluation**:

Anthocyanins give visible red to blue – violet colours.
Compounds of Croci stigma give yellow colours.

# QUINONES




## PROPERTIES, EXTRACTION, SEPARATION AND CHARACTERIZATION

The fundamental properties of quinones are their facile interconversion to hydroquinones (which makes them mild oxidation reagents), and their tendency to add nucleophiles.

Free quinones are practically insoluble in water, can be extracted by the common organic solvents, and their separation requires the common chromatographic techniques. **Benzoquinones** and **naphthoquinones** can be steam distilled. Their stability is fair, but the formation of artefacts is always possible, for example the oxidation by silica gel of 7-methyljuglone to methylnaphtharizin and to dimers, or the methoxylation of naphthoquinones by methanol.

Glycoside extraction is achieved with water or with rather dilute hydroalcoholic solutions. Recovering the reduced forms (quinols, anthrones) is delicate: working at low temperature, away from light, and under nitrogen is required to avoid their spontaneous oxidation during the extraction.

Various color reactions can be used to characterize quinones. The main one is ***Bornträger's*** reaction, obtained by dissolving the quinone in alkaline aqueous medium: the solution develops a vivid color which ranges, depending on the structure and the substituents of the quinone, from orangy-red to purplish-violet. This reaction is also used to visualize TLC plates. In the specific case of **1,8-dihydroxyanthraquinones**, the reaction with magnesium acetate is used very often: it leads to stable colors.

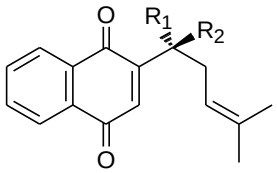
## BIOLOGICAL PROPERTIES AND USES OF QUINONE-CONTAINING DRUGS

Natural benzoquinones in the strict sense of the term have no therapeutical application. 1,4-benzoquinone (i.e., hydroquinone) occurs as a glycoside, namely **arbutin**, and that this molecule possesses strong urinary antiseptic properties. Synthetic hydroquinone has dermatological and industrial (photography) applications.

Many naphthoquinones are antibacterial and fungicidal. The nucleophilicity of these molecules also explains their cytotoxicity. Antiprotozoal and antiviral activities have been described, and several molecules in the group have non-trivial toxicity. Currently, no natural naphthoquinone is marketed for therapy, and only a very limited number of drugs containing them remain in use to produce galenicals (for example *Drosera* sp.).

Drugs containing **1,8-dihydroxyanthraquinone** derivatives have laxative properties, and have been prized for this activity for centuries *(Cassia, Rhamnus),* or even millennia *(Rheum).* They continue to be widely used.

For a long time, some **quinone-containing drugs** had been prized as **dyes**. These included vegetable drugs containing anthraquinones, such as madder root *(Rubia tinctorium*, Rubiaceae), or containing naphthoquinones, such as alkanna root (= alkanet = Anchusae Radix) *(Alkanna tinctoria*, Boraginaceae): the former provides chiefly **alizarin** (the aglycone of ruberythric acid), and the latter provides chiefly **alkannin**. Cochineal, a coloring currently authorized (Eur. id. code **E120),** is traditionally extracted from the desiccated females of a Central American hemipter, *Dactylopius coccus = Coccus cacti*, which contain approximately 10% of a tetrahydroxylated anthraquinone, carminic acid.




**$R_1$ = H, $R_2$ = OH: Alkannin**
**$R_1$ = OH, $R_2$ = H: Shikonin**

**(*R*)-Shikonin** [an isomer of **(*S*)-alkannin**] is found in an oriental Boraginaceae *(Lithospermum erythrorhizon*), is currently produced by tissue culture, and was marketed as a coloring in cosmetology.

## NAPHTHOQUINONE-CONTAINING DRUGS

Naphthoquinones are yellow or orangy pigments essentially from plants, and are characteristic of some Angiosperm families, including Ebenaceae, Droseraceae, and Bignoniaceae. They are almost always 1,4-naphthoquinones, and they are, in very rare cases, 1,2-naphthoquinones. The pharmaceutical interest of this group is very limited.

**Plants rich in Naphthoquinone**

| ***Latin Name*** | English Name | Turkish Name | **Family** |
|---|---|---|---|
| ***Drosera spec.*** | Carnivo | Böcek kapan bitkiler | **Droseraceae** |
| ***Juglans regia*** | Walnut | Ceviz | **Juglandaceae** |
| ***Lawsonia inermis*** | Henna | Kına | **Lythraceae** |

| ***Latin Name*** | Naphthoquinone constituents | **Uses** |
|---|---|---|
| ***Drosera spec.*** | Plumbagin | Antispasmodic, antibacterial |
| ***Juglans regia*** | Juglone | Astringent (external use) |
| ***Lawsonia inermis*** | Lawsone | Skin ailments, burns, wounds, antiepileptic, abortifacient, coloring and cosmetic ingredient |

## Quinons: 1,4-Naphthoquinones

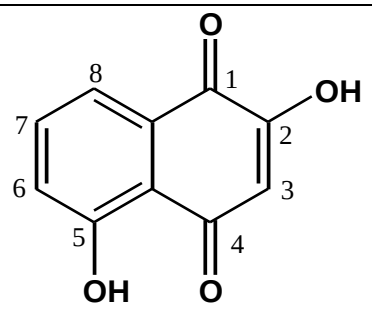
### Plants Containing 1,4-Naphthoquinones

| | |
|---|---|
| ***Juglans regia***<br>Persian walnut, Ceviz<br>**Juglandaceae** | |
| **Carya illinoensis,**<br>Pecan, Pika Cevizi<br>**Juglandaceae)**<br>*Syn. Juglans pecan* | |
| **Lawsonia inermis**<br>Henna plant, Flower of paradise<br>Kına<br>**Lythraceaea** | |
| **Drosera & Dionaeae spec.**<br>Böcek kapan bitkiler<br>**Droseraceae**<br>**Drosera stolonifera**<br>**(google.com.by)** |  |

## NAPHTHOQUINONES

### Lawsone

*Reference*: **CLASSICS IN SPECTROSCOPY** – Isolation and Structure Elucidation of Natural Products, **Stefan BERGER & Dieter SICKER, WILEY-VCH, Weinheim, 2009**

| | |
|---|---|
| **2-Hydroxy-1,4-naphthalenedione**<br><br>Plant: The leaves of the henna plant (Kına)<br><br>***Lawsonia inermis*** L. (Lythraceae)<br><br>$C_{10}H_6O_3$, Mw 174.15,<br><br>Orange crystals, mp 193-195 °C |  |

**Caution!**

Lawsone reacts on contact with the skin to form a permanent stain that lasts until the skin is shed. Therefore, it is strongly recommended to work tidily with both aqueous and organic solutions containing lawsone to avoid a tenacious discoloration of the skin lasting several weeks. Wearing resilient protective gloves is advisable. An unwanted fixing of lawsone in the laboratory is very different from henna body art.

**Background**: An exceptionally good dye for proteins

It has firstly been described in 1870 and noticed its remarkable acidity and even named it "Naphthalinsaure" (naphthalinic acid) although they knew it belonged to the quinones. Another expression of this property is the trivial name hennotannic acid. Interesting from the chemist's point of view are the redox properties of naphthoquinones such as lawsone.

The driving force to deal with such naphthoquinone dyes was the upcoming dyestuffs industry. At the end of the 19th century it became the mother of industrial chemistry. About 50 years later, an Italian chemist discovered that the colouring matter in henna leaves had the same structure. He gave the compound the trivial name lawsone due to its origin, the henna plant *Lawsonia inermis* L..

*That it got the name Lawsonia is in turn based upon the fact that the henna plant was named by the great Swedish botanist Carl von Linne (1707-1778), who established the modem binomial nomenclature and is therefore known as the father of modem taxonomy. All plant names which have the abbreviation L. at the end evoke the name Linne. The reason why Linne*

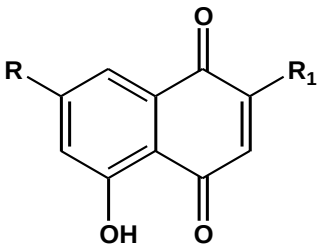
*gave the name Lawsonia to a genus within the family Lythraceae is that he wanted to thank the Scottish doctor of mcdicine Isaac Lawson, one of his investors who supported the publication of Systema Naturae, a book on the hierarchical classification of Nature in three large divisions, namely the plant, the animal and the mineral kingdoms, in 1735. The Latin term inermis means defenceless, i.e. a plant without spines. This is true for the young plant; mature plants have spines and were regarded as a separate species in Linne's times.*

Lawsone occurs in the henna plant leaves in the form of glycosidic precursors that have to be cleaved prior to isolation. It was such henna constituents that were the basis to claim a combination of 1,2,4-trihydroxynaphthalene glycosides and glycosidases as a suitable means for hair dyeing.

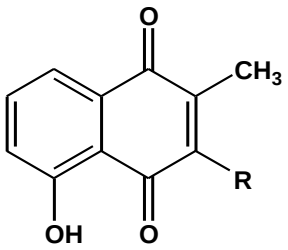
The henna plant is a tall flowering shrub or tree about 5 m in height, native to tropical and subtropical regions of Africa, Asia and northern Australia (tropical savannah, the tropical arid zone, oases in the Sahara).

The practical use of henna leaf powder as a dye for colouring hair and nails and for decoration of parts of the body temporarily is known as Mehndi (also known as Mehandi or Mehendi) traditionally in India, Pakistan, Iran and North Africa. The tradition is very old. Egyptian mummies decorated with henna paintings have been found. Naturally made henna colorations are considered as harmless if they only contain lawsone and no chemical additives. Some native tribes in North Africa apply henna paste on the skin without any decoration as a protection against the sun. This is possible, because lawsone strongly absorbs UV radiation and so do its covalent reaction products with the protein keratin in the skin.

Since ancient times, henna leaves have been used in traditional medicine as an astringent, antiseptic and antipyretic. Henna was used in ancient times also to treat serious diseases (leprosy, smallpox, chickcnpox, tumours) by Arabian doctors. More recently, henna has been investigated and some physiological effects have been confirmed, e.g. bactericidal and fungicidal action (by its tanning effect). Plenna itself is not an allergen, nor could rumours be proved that it might be a carcinogen. The wound-healing process is supported by a henna leaf extract - a fact that was already known to African healers, and was recently confirmed. Recently, lawsone was found to be suitable as a reagent for the detection of latent fingermarks on paper, which is still an extremely important requirement in criminology as contact evidence. Lawsone, in this context, could serve as a substitute for ninhydrin, used hitherto.




**Juglone, R = $R_1$ = H**
**Lawsone, R = H, $R_1$ = OH**
**Methyljuglone, R = $CH_3$, $R_1$ = H**




**Plumbagin, R = H**
**Droserone, R = OH**

## 10a. THIN LAYER CHROMATOGRAPHY OF NAPHTHOQUINONES

**Preparation of Extract**

**Lawsonia inermis**
**Juglans regia (Juglandaceae)**
**Carya**

B. Powdered drug (1 g) is extracted for 15 min with methanol (10 mL) on a water bath; 30 µL of the clear filtrate is used for TLC.

C. Powdered drug (1 g) is distilled with water (10 mL) and 2M $H_3PO_4$ in a 50 mL-flask through a glass pipe into a chilled glass tube until 3 ml distillate has been collected. After cooling, the lipophilic compounds are extracted with 1 mL pentane, 10 µL of this solution is used for TLC.

**Drosera herba, Dionaeae herba**

D. The whole fresh plant is put through a tincture press until 1 ml plant juice has been collected. The juice is diluted with 9 ml $CHCl_3$ and 20 µl is used for TLC investigations.

**Thin-Layer Chromatography (TLC)**

**Reference solution** : 10 mg plumbagin and juglone are dissolved in 1 mL methanol and 10 µL is used for TLC investigation.
**Adsorbent** : Silica gel $60F_{254}$ precoated TLC plates
**Solvent system** : Toluene-formic acid (99:1)
***Naphtoquinone aglycones***
**:** Ethyl acetate-formic acid-acetic acid-water (100:11:11:26)
***Glycosides***

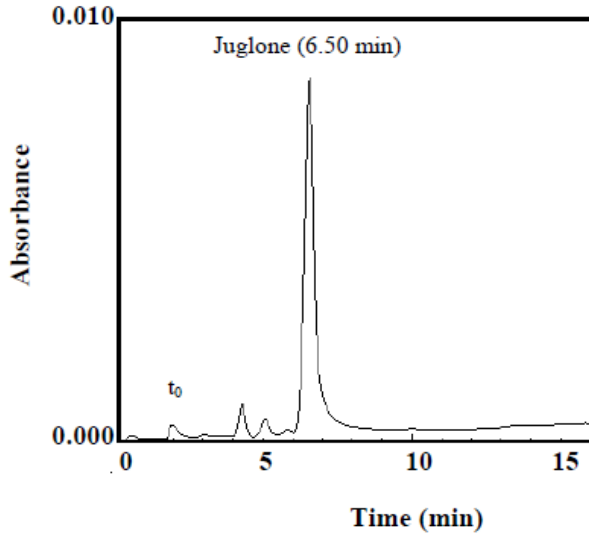
**Detection**

- All naphthoquinones show quenching in UV-254 nm.
- After spraying with 10% methanolic KOH reagent, naphthoquinones show red fluorescence in UV-365 nm and red to red-brown colour in day light.

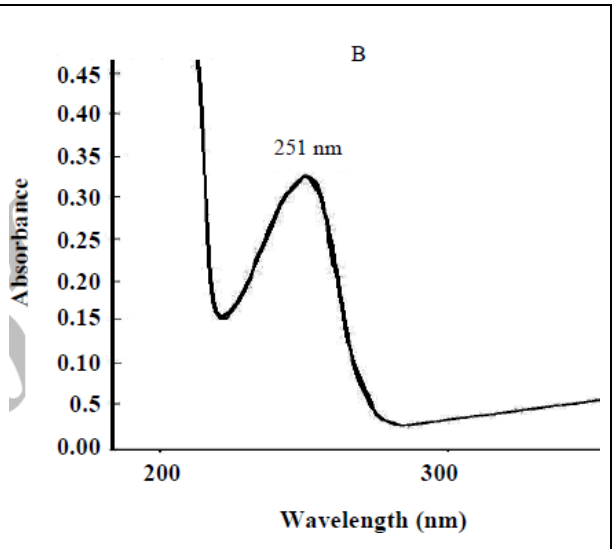
**Plant Drug Analysis: A Thin Layer Chromatography Atlas**
Hildebert Wagner,Sabine Bladt, Springer, Berlin, 1996

## JUGLONE

**Determination of juglone by RP-HPLC**




Chromatogram of chloroform extract from leaves



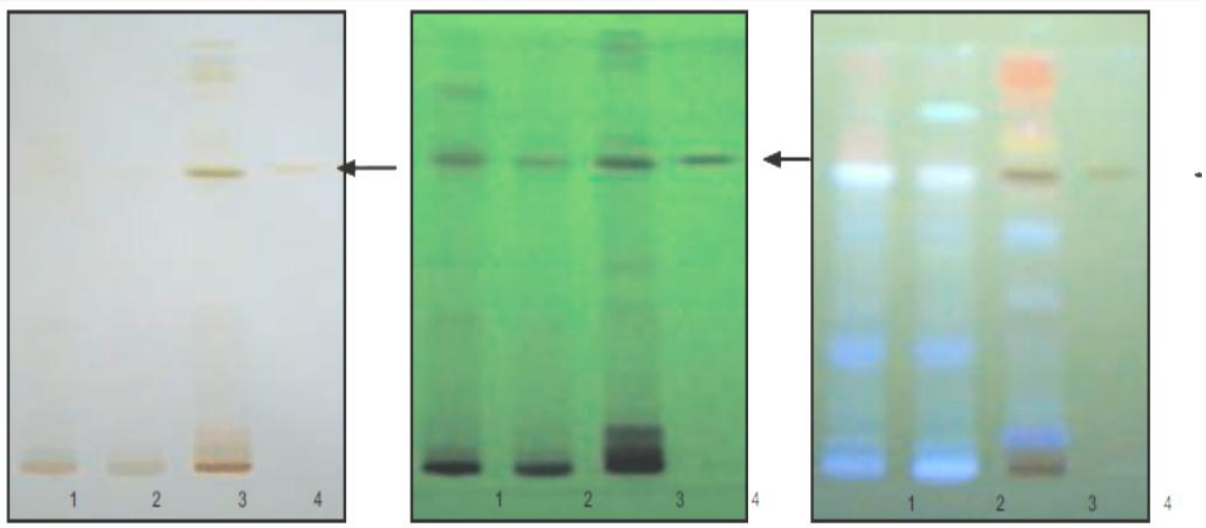

UV Spectrum of juglone.

Ref.: M.R. Hadjmohammadi & K. Kamel, ***Iran J. Chem. Chem. Eng.***, 25, 73 – 76 (2006).

**RP- HPLC Conditions: Mobile phase: 50% Acetonitrile in Water, pH = 4, 30 °C; Flow rate: 1.5 mL/min; Column: 250 x 4.6 mm, µBondapak C-18, 10µm**

## LAWSONE

**TLC of Lawsone**




| Visible | 254 nm | 366 nm |
|---|---|---|

TLC of callus powder and leaf powder of ***Lawsonia inermis***. **1**. Young leaf callus powder; **2**. Old leaf callus powder; **3**. Leaf powder of field grown plant; **4**. Standart **LAWSONE (⟵)**

*Reference*: S.S. Phirke, M. Saha, N. Chandra. ***ASIAN J. Exp. Biol. Sci. SPL.*** 118 – 120 (2010)

**Solvent system** : Toluene-Ethylacetate-Acetic acid (5:4:1)

## Additional information for extraction of lawsone:

*Reference*: **CLASSICS IN SPECTROSCOPY** – Isolation and Structure Elucidation of Natural Products, **Stefan BERGER & Dieter SICKER, WILEY-VCH, Weinheim, 2009**

**Extraction of *Lawsonia inermis***

Extraction of Lawsone from the leaf of **Lawsonia inermis** (Henna, Kına): Lawsone occurred in glycosidic form in the leafs. Glycosides have to be cleaved prior study. For this purpose, hydrolysis is required which can be achieved by acidic hydrolysis or keeping in hot water for a long time (24 hrs).

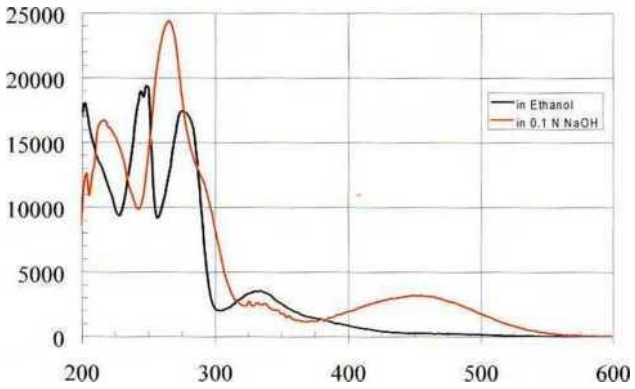
Dried and powdered leaves contain intact glycosidase which is able to split the glycosidic bond when brought int contact with hot water. Therefore the henna leaf powder suspension is stirred for several hours in hot water at 70 °C. Lawsone is not readily soluble in water but acidic. At the end of hydrolysis $NaHCO_3$ is added to make the aqueous phase weakly basic (pH 7.5). This process brings lawsone into solution before the suspension is filtered. The filtrate is then acidified and lawsone is extracted into diethyl ether.

The colour of lawsone differs from an intense orange-yellow to dark red-brown. On the TLC plate in the eluent mentioned in all cases an intense dark orange spot can be observed.

**Method**

Powder of dried henna leaves (5 g) is placed in a large beaker and distilled water (200 mL) is added together with a magnetic stirring rod. The suspension is stirred on a magnetic stirrer with heating while the temperature is kept at 70 °C. After 45 min, the colour of the green suspension turns to brown. After 4 h, solid NaHCO, (1 g) is added. The suspension is filtered by gravity overnight over three large glass funnels with filter paper (diameter 30 cm). This kind of filtration is slow but works reliably. Attempts to force the pace by suction filtration are not advisable because then colloidal particles will rapidly plug the pores of the filter. The filtrates are combined and acidified to pH 3 by addition of 0.12 M HC1. The brown extract undergoes a clarification in this step and turns slightly cloudy. The swollen plant material is discarded. The filtrate is extracted with diethyl ether (4 x 25 mL). In the final extraction, the ether turns to a very pale yellow, indicating the end of extraction. The aqueous phase does not change its brown colour during extraction but turns clear and can be discarded after the extraction. The combined ethereal phases are washed with water (3 x 10 mL) and dried over $MgS0_4$. The ether is removed completely in vacuo to leave a reddish brown solid as crude product.

**UV Spectrum of Lawsone**



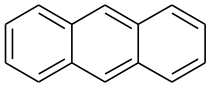

**UV Spectrum of Lawsone in Ethanol and 0.1 N NaOH.**

The UV spectrum of lawsone in ethanol is of course similar to that of 1.4-naphthoquinone, but the main band of 1.4-naphthoquinone at 245 nm is split in lawsone into two *n-n** transitions at 248 and 276 nm. Remarkable is the long tail of the band at 334 nm reaching far into the visible region, which is responsible for the yellow'ish colour of lawsone.

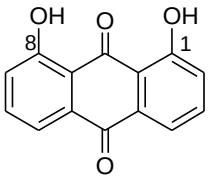
# ANTHRONOIDS, ANTHRAQUINONES

The drugs in this group are characterized by the presence phenolic and glycosidic compounds, derived from anthracene and have variable degree of oxidation (anthrones, anthronols, athraquinones): They are the anthraquinone glycosides. These molecules have in common a double hydroxylation in the C(1)- and the C(8)-positions.

Anthranoid derivatives are used all over the world as a treatment for constipation. These compounds are present in several drugs of plant origin, especially as O- or C-glycosides. Besides featuring different substituents, the aglycone might consist of an anthraquinone, an anthrone or a dianthrone.



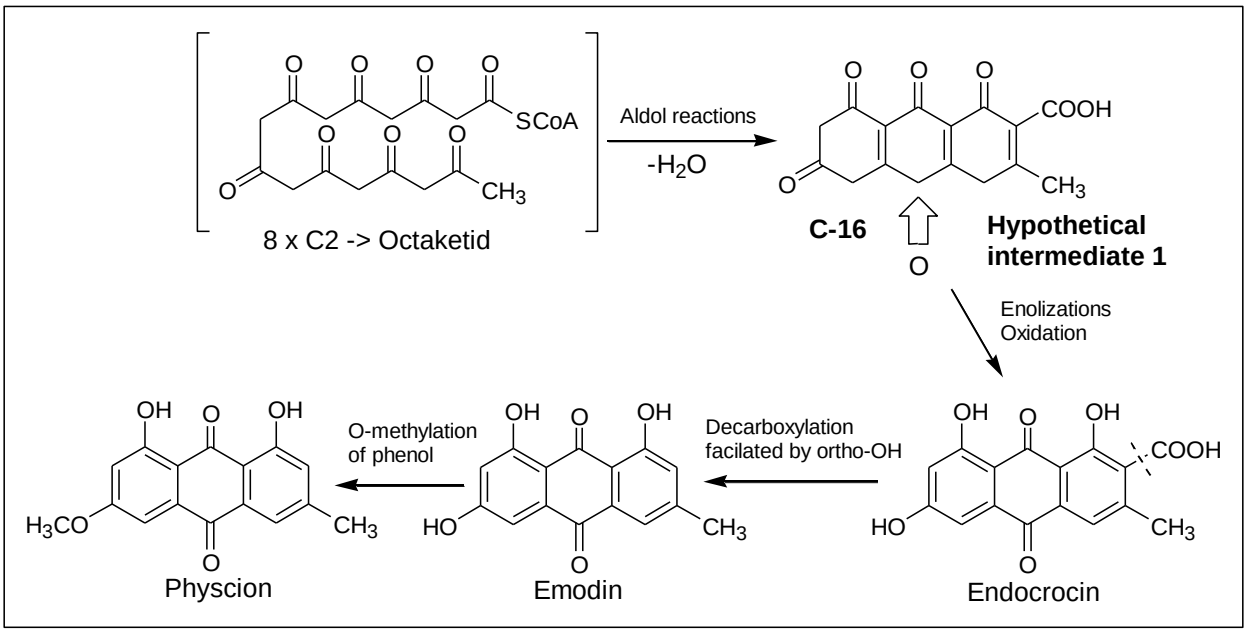
Anthracene




1,8-dihydroxy-anthraquinone
**(Danthron)**

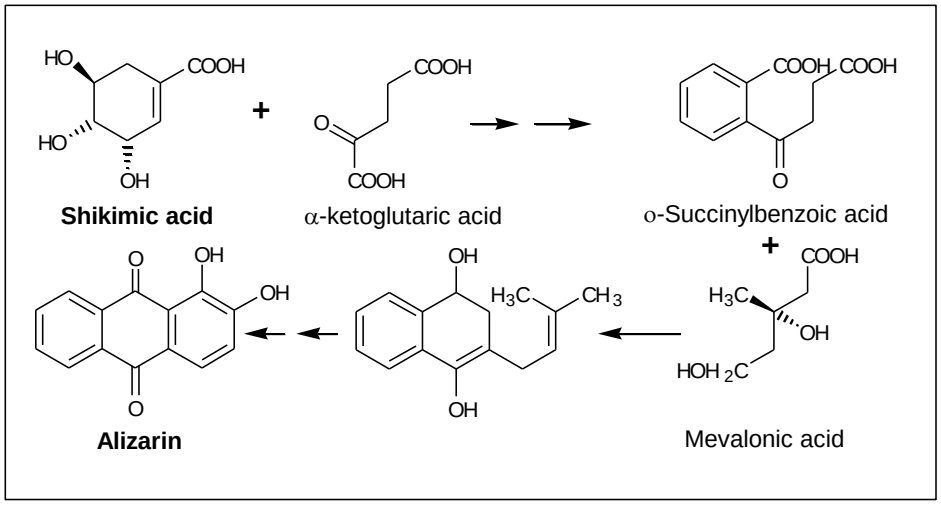
**Biosynthesis of anthronoids**

**a. Acetate/malonate pathway:** Anthraquinone derivatives are excellent examples of acetate-derived structures. **Endocrocin** found in species of *Penicillium* and *Aspergillus* fungi is formed by folding a polyketide containing eight C2 units to form the periphery of the carbon skeleton. Three aldol-type condensations would give a **hypothetical intermediate 1**, and, except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions. The additional carbonyl oxygen must be introduced at some stage during the biosynthesis by an oxidative process. **Emodin**, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. This is facilitated by the adjacent phenol function. *O*-Methylation of emodin would then lead to **physcion**.




*Medicinal Natural Products.* Paul M Dewick, John Wiley & Sons, Ltd., New York 2002.

**b. Shikimate /** 2-oxoglutarate **/ isoprenoid pathway:**

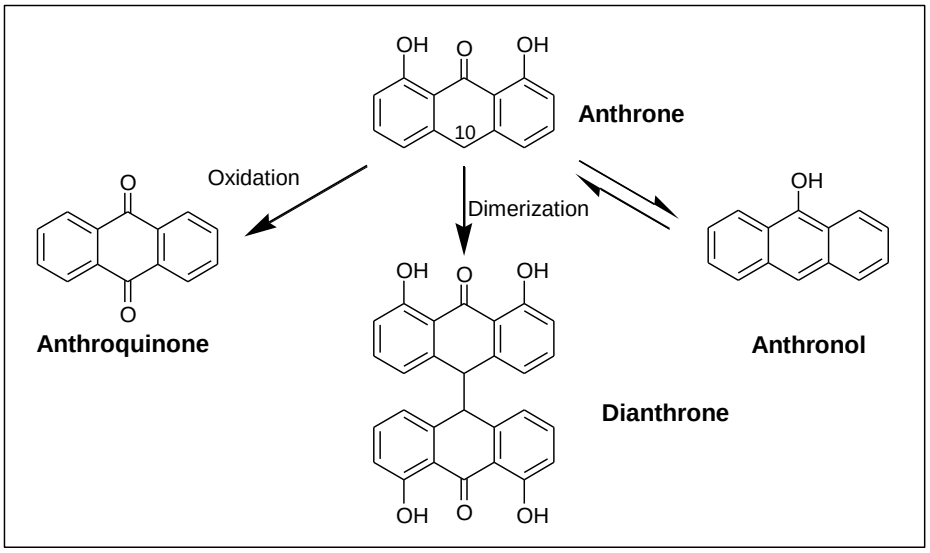



Gunnar Samuelsson, **Drugs of Natural Origin –**A Textbook of Pharmacognosy, Apotekarsocieteten, Kristianstadt, Sweden 2004.

The existence of this pathway was first demonstrated for alizarin and purpurin carboxylic acid, both of which are found in *Rubia tinctorium* (Rubiaceae). In this reaction, shikimate reacts with -ketoglutaric acid. The product, -succinylbenzoic acid together with mevalonic acid (formed from acetate) gives an intermediate that yields alizarin by ring closure.

**The Structure of Anthranoids**:

The degree of oxidation varies. In anthrones (10-*H*-anthracene-9-ones), carbon 10 is methylene carbon. Depending on the pH, these anthrones can occur alongside their tautomeric forms, the anthranols. In practice, anthrones and anthranols are often designated by the term "reduced forms", and anthraquinones by that of "oxidized forms".

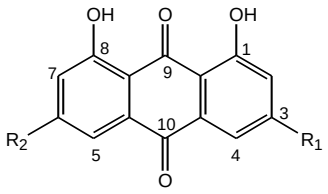



Interrelationships of anthraquinone derivatives

Under some conditions (during the drying of drugs), anthrones may combine into dianthrones. These are referred as homo- or heterodianthrones, depending on whether the constituent anthrones in dimer are identical or different, respectively. Anthrones are unstable. Therefore, the free aglycones that occasionally occur in the drugs are always anthraquinones. The reduced forms exist only in the combined state, in other words as glycosides.

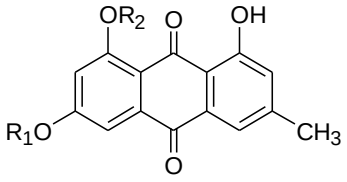
## ANTHRAQUINONE-CONTAINING DRUGS

### Anthraquinones



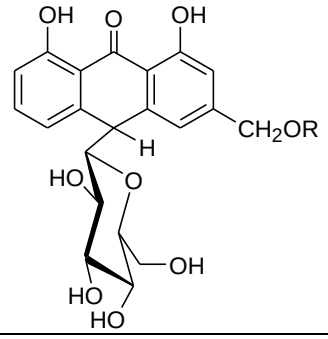

| $R_1$ | $R_2$ | Anthraquinones |
|---|---|---|
| $CH_3$ | OH | Emodin |
| $CH_3$ | $OCH_3$ | Physcion |
| $CH_3$ | H | Chrysophanol |
| $CH_2OH$ | H | Aloe-emodin |
| COOH | H | Rhein |

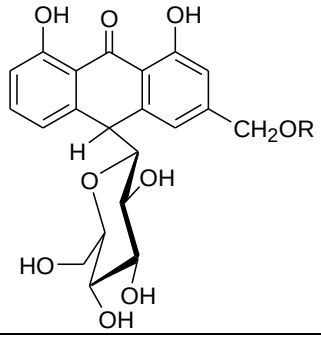
### Anthraquinone Glycosides




| $R_1$ | $R_2$ | Anthraquinone Glycosides |
|---|---|---|
| Glucose | Rhamnose | **Glucofrangilin A** |
| Glucose | Apiose | **Glucofrangilin B** |
| H | Rhamnose | **Frangilin A** |
| H | Apiose | **Frangilin B** |

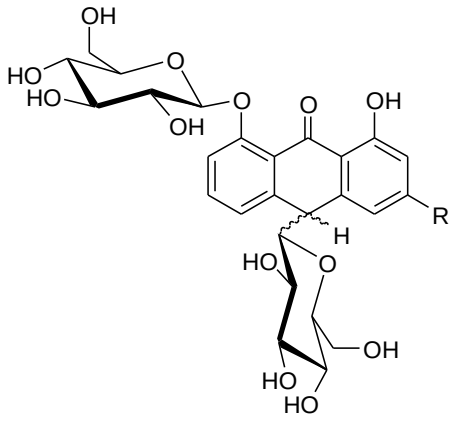
### Anthrone C-Glycosides



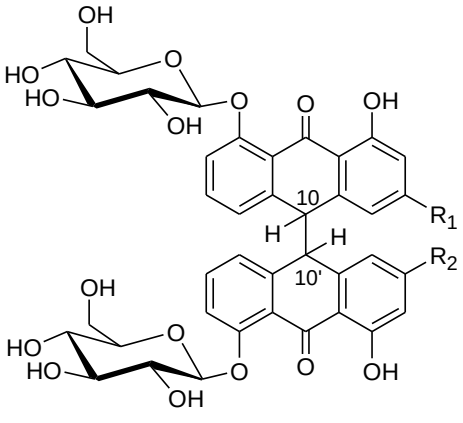




| R | Glycosides |
|---|---|
| H | **Aloins A, B** |
| Rhamnose | **Aloinosides A, B** |

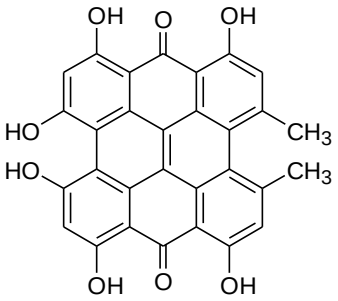
### Anthrone C-Glycosides



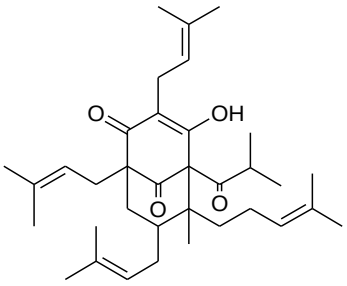

| R | Glycosides |
|---|---|
| $CH_2OH$ | Cascarosides A & B |
| $CH_3$ | Cascarosides C & D |



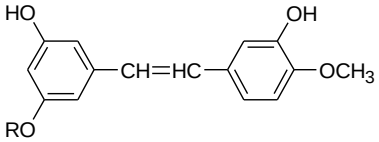

**Sennoside A**: $R_1$, $R_2$ = COOH (+)-form
**Sennoside B**: $R_1$, $R_2$ = COOH Mesoform
**Sennoside C**: $R_1$ = COOH $R_2$ = $CH_2OH$ (+)-form
**Sennoside D**: $R_1$ = COOH $R_2$ = $CH_2OH$ Mesoform




**Hypericin**




**Hyperforin**




Rhapontigenin R = H

**Rhaponticoside R = Glucose**

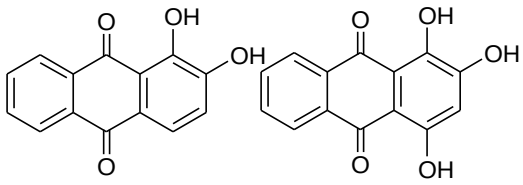
**Anthraquinone containing Herbal Drugs**
**LAXATIVE 1,8-Di-HYDROXYANTHRAQUINONE GLYCOSIDES**

Botanical distribution of the species containing 1,8-dihydroxyanthraquinone glycosides is very limited [Liliaceae, Polygonaceae, Rhamnaceae and Caeselpiniaceae (Fabaceae)].

| **Families** | **Drugs** | ***Plants*** | **Main antrhronoids** |
|---|---|---|---|
| Liliaceae | **Aloe extractum** | *Aloe species* | Aloin, Aloinosides A&B |
| | **Curacao Aloe** | *Aloe barbadensis* | Aloins |
| | **Kap Aloe** | *Aloe capensis* | Aloins, Aloinosides A&B, Aloesins A/B |
| | **Socotro Aloe** | *Aloe perryi* | Aloins, Aloinosides A&B, Aloesines A&B |
| Rhamnaceae | **Rhamni purshiani Cortex** | *Rhamnus purshinanus* | Cascarosides A&B&C&D |
| | Cascarae sagradae cortex | | Aloins and Desoxyaloin |
| | **Frangulae cortex** | *Rhamnus frangula* | Glucofrangulins A&B<br>Frangulins A&B)<br>Emodin mono- and diglucosides, physcion and chrysophanol glycosides |
| | **Rhamni cathartici fructus** | *Rhamnus catharticus* | Frangulin and glucofrangulin (trace amounts) |
| Polygonacea | **Rhei radix** | *Rheum palmatum*<br>*Rheumn officinale* | Physcion-, chrysophalol-, rhein mono and diglucosides<br>Rhein, physcion, chrysophanol, emodin<br>Rheinosides, Sennidins |
| Fabaceae (Caesalpiniaceae) | **Sennae folium** | *Cassia senna* | Sennosides |
| | | *Cassia angustifolia* | Sennosides |
| | **Sennae fructus** | *C. senna, C. angustifolia* | Sennosides A – D |
| Hypericaceae | **Hyperici herba** | *Hypericum perforatum* | Hypericin<br>Hyperforin |

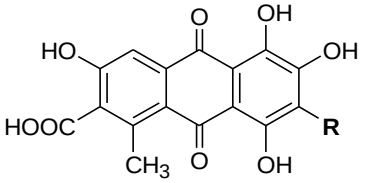
**ANTHRANOIDE COLORANTS** (ANTHRAQUINONES AS NATURAL COLORING AGENTS)

**Alizarin**
red from madder, *Rubia tinctorum* (Rubiaceae) occurs in nature as (glycoside) called ruberythrinic or ruberythric acid.
*Galium mollugo,* White Bedstraw, Bedstraw roots, were used by native Indians to obtain a red colour, The root of this plant, were an important source of the dye alizarin. *Galium Odoratum,* Sweet Woodruff is similar to G. Mollugo.




1,2-dihydroxyanthraquinone

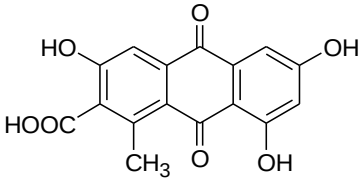
**Carminic acid** is an anthraquinone glycoside that occurs naturally in some scale insects, such as the cochineal and the Polish cochineal. Carminic acid is the colouring agent in carmine.
**Cochineal** is a natural dye substance that comes from dried crushed bodies of female insects, *Coccus cacti* or *Dactylopius coccus*, found on prickly pear cacti (Mexico, Central America). Cochineal can be used with or without mordants and produces reds, pinks and purples.



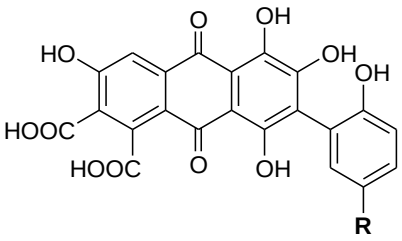

**R** = **H**, Kermes (dye), **kermesic acid** red (λmax 498 nm) violet-red in $H_2SO_4$, blue in boric acid, violet on alkali
**R** = **Glucose**, **Carminic acid** black red in water, yellow to violet in acids

**Kermesic acid** is obtained from the lac insect, *Laccifer lacca* (super family: Coccoidea); a parasite of a large number of trees.

**Lac**, yellow-red colorant, is obtained from the secretions of an insect found in India (*Coccus laccae*).
**Laccaic acids A, B, C** and **D**, laccaic acid A being the most abundant. Crimson dye.



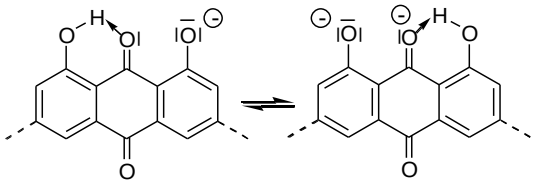

**Laccaic acid D**




**R =** -$CH_2CH_2NHCOCH_3$, **Laccaic acid A**
**R =** -$CH_2CH_2OH$, **Laccaic acid B**
**R =** -$CH_2CH(NH2)COCH_3$, **Laccaic acid C**

**Color Reaction of 1,8-dihydroxyanthraquinone**

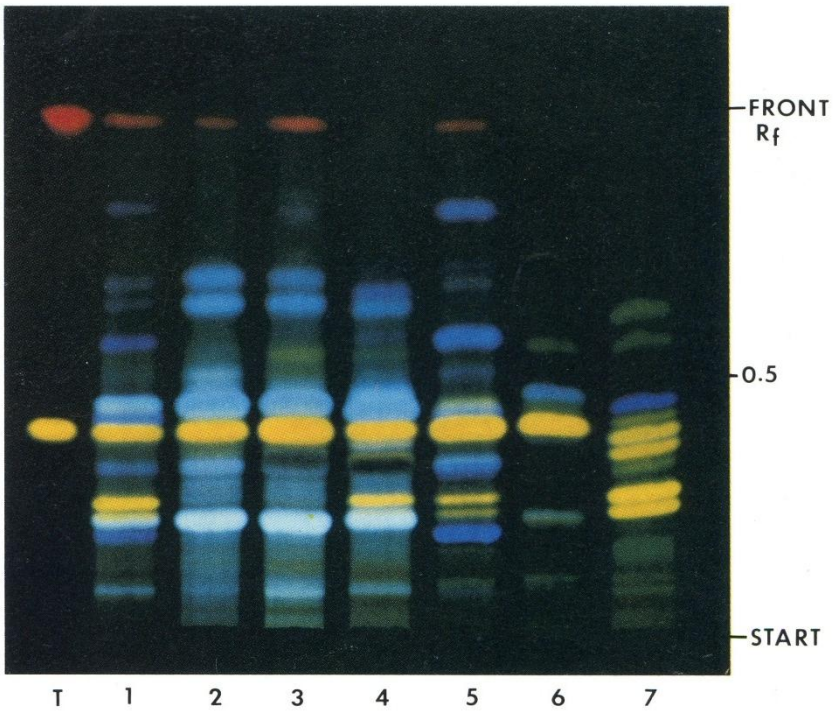
**Bornträger Reaction**:
1,8-dihydroxyanthraquinone-aglycones give a red color in alkaline aqueous medium: The solution develops a vivid color which ranges, depending on the structure and the substituents of the quinone, from orange-red to purplish-violet. This reaction is also used to visualize TLC plates.




*Tautamere border structures*

**Magnesium acetate**: The reaction of 1,8-dihydroxyanthraquinones with magnesium acetate is used very often and leads to stable colors.

## 10 b TLC of Anthraquinone-containing Drugs – 1

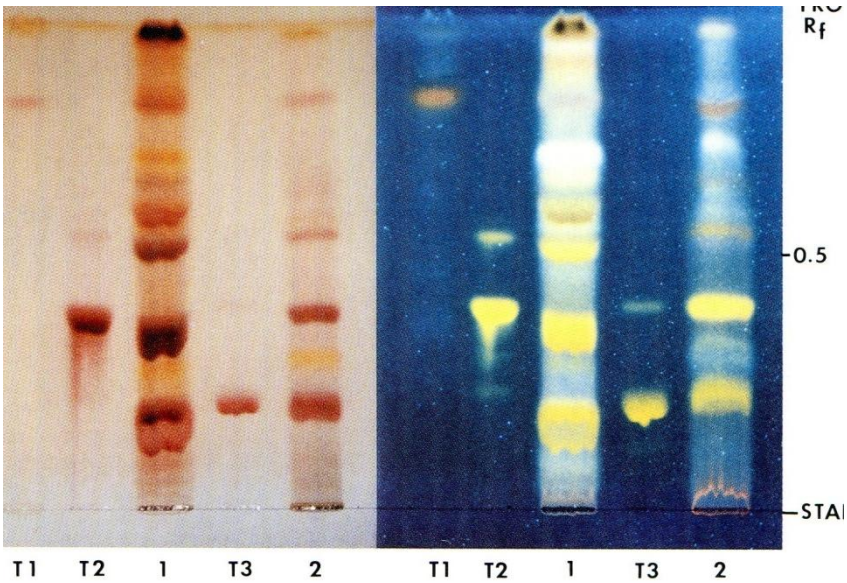



**Herbal Drugs**
**1. Aloe capensis**
(Kap Type A)
**2. Aloe capensis**
(Kap Type B)
**3. Aloe barbadensis**
(Curacao-Aloe)
**4. Aloe perryi**
(Socotro Aloe)
**5. Aloe** (Kenia)
**6. Aloe** (Uganda)
**Aloe** (India)

**Test solution:**
Aloin (Rf 0.45 – 0.50)
Aloe-emodin (Rf front)

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate – MeOH – Water (100:13.5:10)
**Detection**: KOH R.

**A** **B**




**Chromatograms A&B**

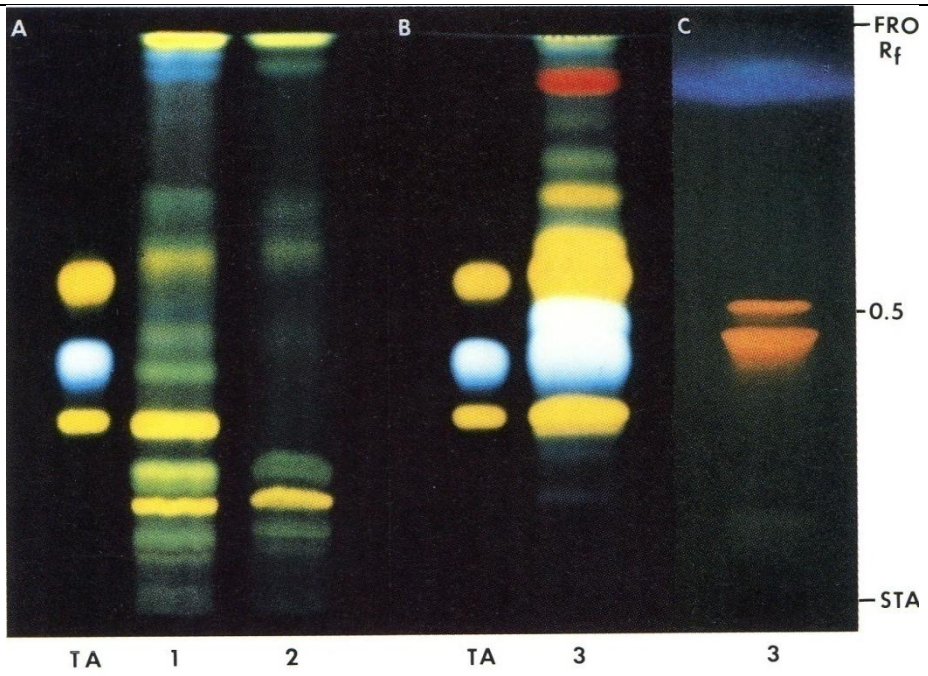
**Herbal Drugs**
**1.Sennae folium**
**2 . Sennae fructus**

**Test solutions**
T1. Rhein
T2. Sennoside A
T3. Sennoside B

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** n-Propanol – Ethylacetate – Water (40:40:30) (**Chromatograms A&B**)
**Detection**: $HNO_3$ R. – KOH R., day light (**Chromatogram A**), UV-365 nm (**Chromatogram B**)
$HNO_3$ R.: Conc. $HNO_3$
KOH R.: 5% KOH solution in EtOH.
Uses: After spraying of conc. $HNO_3$, plates are heated 15 min at 120 °C , than KOH R. is sprayed on to TLC plates. Anthroquinones are seen as red-brown colors under day-light, which are observed as yellowish-brown under UV – 365 nm.

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen

## TLC of Anthraquinone-containing Drugs – 2



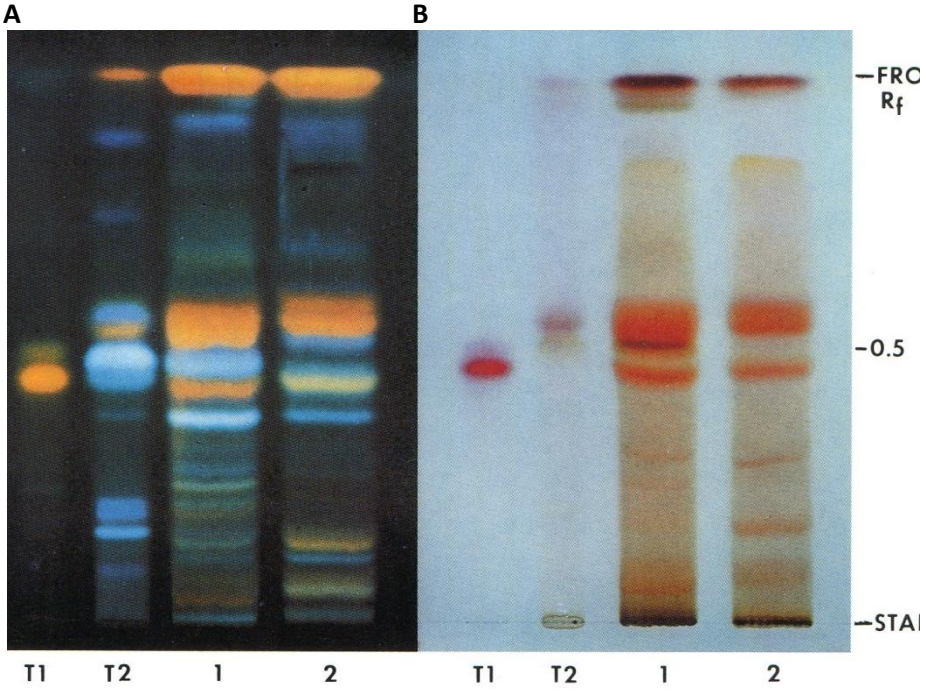

**Herbal Drugs**

1. **Sennae folium**
2. **Sennae fructus**
3. **Hyperici herba**

**Test solutions**

T1. Rhein
T2. Sennoside A
T3. Sennoside B
TA.
Rutin (RF ca. 0.4)
Chlorogenic acid (Rf ca. 0. 45),
Hyperoside (Rf 0.6)

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate – Formic acid – Acetic acid - Water (100:11:11:27) Chromatograms A&B
Mobile phase: Toluol – Ethylformat – Formic acid (50:40:10)
**Detection**: KOH R. (Chromatograms A&B); Pyridin (10% in EtOH) (Chromatogram C)




**Herbal Drugs:**
**1. Rhei rhapontici r.**
**2. Rhei palmati r.**

**Test Solutions**

T1. Rhein
T2. Rhapontizin &

Desoxyrhapontizin
(Rf ca. 0.45 – 0.55)

**Adsorbent:** Silica gel $GF_{254}$
**Mobile phase:** Ethylacetate – MeOH – Water (100:13.5:10) (Chromatograms A&B)
**Detection**: KOH R., day light, UV-365 nm (Chromatograms A&B)
KOH R.: 10% KOH in EtOH.

Ref. H. Wagner, S. Bladt, E.M. Zgainski, Drogen analyse: DC Analyse von Arzneidrogen

**REPORT**

| **NEPHAR 302 PHARMACOGNOSY I LABORATORY** | **Microscopy Lab. No:** |
|---|---|
| **Name Surname :**<br>**Number :** | **Microscope No :**<br>**Assistant :** |
| **Subject**: | Date: |
| **Drug:**<br>**Plant:**<br>**Family:**<br>**Reagent:**<br>**Scale:** | **<u>Organoleptic properties</u>**<br>**Colour:**<br>**Odour:**<br>**Taste:**<br>**Appearance:** |
| **Drawings:** | |
| Observations: | |

*Form A*

**REPORT**

| **NEPHAR 302 PHARMACOGNOSY I LABORATORY** | **Laboratory for Natural Products Chemistry - Phytochemistry** |
|---|---|
| **Name Surname :**<br>**Number :** | **The Name of Test/Assay:**<br><br>**Date:** |
| **Group:** | **Asistant:** |
| **Plant Material** | |

**REPORT**

| **NEPHAR 302 PHARMACOGNOSY I LABORATORY** | **Laboratory for Natural Products Chemistry - Phytochemistry** |
|---|---|
| **Name Surname :**<br>**Number :** | **The Name of Test/Assay:**<br><br>**Date:** |

*Form B 2/2*