King Saud University

Collage Of Pharmacy

Pharmacognosy dept.

**434 PHG**

**Practical Course**

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Lab-1

**Herbarium**

**Definition:**

It is a collection of preserved [plant](http://en.wikipedia.org/wiki/Plant) specimens. These specimens may be whole plants or plant parts: these will usually be in a dried form, mounted on a sheet, but depending upon the material may also be kept in alcohol or other preservative.

**Specimen preservation:**

* To preserve their form and color, plants collected in the field are spread flat on sheets dried.
* The specimens, which are then mounted on sheets of stiff white paper, are labeled with all essential data, such as date and place found, family, species, description of the plant, altitude. The sheet is then placed in a protective case.
* Certain groups of plants are soft, bulky, or otherwise not amenable to drying and mounting on sheets. For these plants, other methods of preparation and storage may be used. For example, [conifer cones](http://en.wikipedia.org/wiki/Conifer_cone) and [palm](http://en.wikipedia.org/wiki/Arecaceae) fronds may be stored in labeled boxes.
* Representative flowers or fruits may be pickled in [formaldehyde](http://en.wikipedia.org/wiki/Formaldehyde) to preserve their three-dimensional structure.

**Uses:**

1. Herbaria are essential for the study of [plant taxonomy](http://en.wikipedia.org/wiki/Plant_taxonomy), the study of geographic distributions, and the stabilizing of nomenclature.
2. Herbaria used to catalogue or identify the [flora](http://en.wikipedia.org/wiki/Flora) of an area.
3. Herbaria also preserve an historical record of change in [vegetation](http://en.wikipedia.org/wiki/Vegetation) over time.

**Procedures for extraction and isolation of plant constituents**

The appropriate method of extraction depends on number of factors

1. Texture
2. Water content
3. Type of substance that is being isolated
4. Purification depends on the selectivity of solvent

**Preparation of the plant material before extraction**

The plant material must be prepared before extraction by cutting or crushing, ranging from coarse, medium to fine powder

**General procedures**

**1-Infusion**

Plant material is placed in a pot and wetted with cold water, boiling water is poured over it, and left to stand, covered with lid (lift for 15’) then poured

**2-Maceration**

Used for water soluble active constituents.

It consist of macerating the plant material in cold water for several hours

**3-Digestion**

This method is suitable for hard barks or woods which are difficult for water to penetrate.

**4-Decoction**

It consists of boiling plant material for ten minutes or if boiling water is poured over it and allowed to stand for thirty minutes.

**5-Continuous hot extraction method**

This procedure is considered as the most common method used for the extraction of organic constituents from dried plant tissues

The powdered material is continuously extracted in a soxhlet apparatus with a range of solvents.

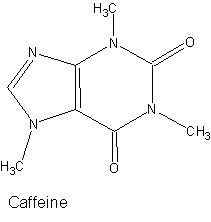
**6- Solvent –solvent precipitation**

The extract dissolved in a suitable solvent, is mixed with a less polar but miscible solvent causing the selective precipitation of the less soluble plant constituents.

**7- Liquid –liquid extraction**

The solute molecules are partitioned between two immiscible solvents. The amount of solute in each phase will depend upon their relative solubility in each solvent. which in turn is related to their polarity.

**Extraction of Caffeine from Tea Leaves**

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**Caffeine** is a naturally occurring **xanthine alkaloid** found in coffee, tea, Cocoa.

The leaves may be fermented or left unfermented. Fermented teas are referred to as **black tea**, unfermented teas as **green tea**, and partially fermented teas as **oolong tea**.

Caffeine is chemically 1,3,7-trimethylxanthine (C8H10N4O2).

Caffeine acts as a stimulant. It stimulates the heart, respiration, the central nervous system, and is a vasodilator (relaxes the blood vessels) as well as a diuretic (increases urination).

**Principle of the experiment:**

The technique used to separate an organic compound from a mixture of compounds is called **Extraction.** The solution of these dissolved compounds is referred as the **Extract.**

Tea leaves consist mostly of cellulose, caffeine, tannins and a small amount of chlorophyll. Here the organic solvent chloroform is used to extract caffeine from aqueous extract of tea leaves because caffeine is more soluble in chloroform (140 mg/ml) than it is in water (22 mg/ml). However, the tannins that are slightly soluble in the chloroform can be eliminated by converting it to their salts (phenolic anions by adding sodium carbonate).

**Procedure**

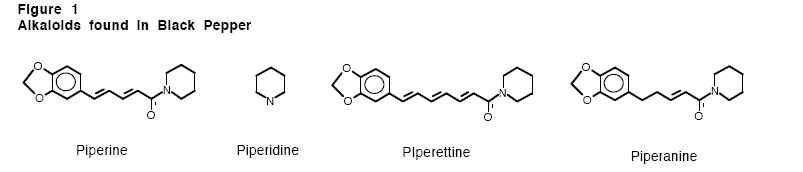
1. Place 30 g of the tea leaves or tea bags in a 500mL beaker. Add 250mL of water and 5g of sodium carbonate and stir the contents of the beaker with a glass rod.
2. Boil the beaker over direct flame for 20 minutes.
3. Ensure separatory funnel valve shut. Using an ordinary funnel, pour the tea into the separatory funnel.
4. Extract the caffeine with four successive 25-mL portions of chloroform. Chloroform and the tea mixture can form an emulsion difficult to separate if allowed to be agitated, so slowly pour the into the separatory funnel.
5. Pass the lower layer from the separatory funnel through a drying agent (such as Na2SO4) through a conical funnel lined with filter paper.
6. Evaporate the combined chloroformic extract to obtain a crude product (use a hot plate).
7. With a spatula, remove the solid left behind and mass it. This solid is principally caffeine.

Lab-2

**Isolation of Piperine from Black Pepper**

Piperine can be isolated in good yield from ground black pepper ***Piper nigrum***, which is made up of 5-9% of alkaloids that also include **piperidine, piperettine and piperanine.**

Historically, pepper has been thought to cure many illnesses such as cancer, malaria, and cholera; however, today it is most commonly used as a food additive.

**Piperine** is tasteless, but its stereoisomer, **chavicine**, is the active ingredient in black pepper that provides its characteristic taste. Loss of pungency during storage of black pepper is attributed to the slow isomerization of chavicine into piperine.

**Principle:**

Piperine is extracted from black pepper by ethanol using a soxhlet extraction apparatus. The piperine is purified by recrystallization

**Materials**

• soxhlet extractor • black pepper

• 95% ethanol 10% alcoholic KOH

**Procedure:**

1. Grind 10 g black pepper to a coarse powder.
2. Extract the ground pepper with 200 mL 95% ethanol in a soxhlet extraction apparatus for 3hours. (The ground pepper is placed in the thimble and the ethanol in the round-bottomed flask.
3. Allow the solution to cool and filter through Whatman filter paper.
4. Concentrate the solution to remove most of the ethanol solvent. The final volume should be about 5mL.
5. Add 10mL 10% alcoholic KOH to the residue and let stand 1 hour.
6. Decant the solution from the insoluble residue.
7. Allow the alcoholic solution to stand undisturbed overnight; long yellow needles of piperine will be deposited. (The crystals may take 24–48 hours to form.)
8. Collect the yellow needles and wash with a minimum volume of 95% ethanol.
9. Allow the crystals to air dry. Weigh them and determine the melting point. (It should be 125–126°C.).

Lab-4

Analysis of market samples containing volatile oils

**Examples:**

1-Clove.

2-Ginger.

3-Mentha.

4-Thyme.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Thyme | Mentha | Ginger | Clove |  |
| Dried leaves of *Thymus vulgalis* | Dried leaves of *Menthe piperita* | Dried root of *Zingiber officinalis* | Dried flower of *Eugenia caryophyllus* | Origin |
| Labiatae | Labiatae | Leguminosae | Myrtaceae | Family |
| Grayish green | Green | Yellow | Dark brown | Colour |
| Agreeable | Astringent | Pungent | Agreeable | Taste |
| Aromatic | Aromatic | Aromatic | Aromatic | Odour |
| Thymol  Carvacrol | Menthol | Gingerol | Eugenol | Volatile oil |
|  |  |  | With fecl3 gives deep blue colour | Chemical test |

Microscopic examination:

|  |  |  |
| --- | --- | --- |
| Oil gland | Triangular pollen grains | Clove |
| Septate fiber | Starch granules | Ginger |
| Labiaceous hair | Diacytic stomata | Mentha |
| Glandular hair | Covering hair | Thyme |

GLC determination of volatile oils:

Lab-5

**Evaluation methods of market samples containing ALKALOIDS**

Examples:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ipeca | Cinchona | Hyoscyamus | Belladonna | Datura |  |
| Dried root and rhizome of *Cephaelis ipecacuanha* | Dried bark of Cinchona Succirbura | Dried leaves of Hyoscymus niger | Dried leaves of Atropa belladonna | Dried leaves of Datura stramonium | Origin |
| Rubiaceae | Rubiacaceae | Solanaceae | Solanaceae | Solanaceae | Family |
| Brown | Reddish brown | Grayish yellow | green | green | Colour |
| Slight | Slight | Slight | Slight | Slight | Odour |
| Bitter | Astingent | bitter | bitter | bitter | Taste |
| Emetine  Cephaeline | Quinine,  Quinidine | Atropine, Hyoscine,  Hyoscyamine | Atropine, Hyoscine,  Hyoscyamine | Atropine, Hyoscine,  Hyoscyamine | Alkaloids |

Microscopical examination of veg. drugs:

|  |  |  |  |
| --- | --- | --- | --- |
| 3-Non-glandular hair(conical shaped, multicellular,uniceriate). | 2-Cluster layer of Ca.ox in cell. | 1-Anisocytic stomata. | 1-Datura stramonium |
| 3-Glandular hair(multicellular stalk,unicellular head). | 2-Sandy crystals of Ca.ox | 1-Anisocytic stomata. | 2-Atropa belladonna |
| 3-Branched hair(glandular hair). | 2-Ca.ox (Twin prism). | 1-Anisocytic stomata. | 3-Hyoscyamus |
| c- Cork cell. | b- Starch granules. | a- Phloem fibers e`funnel shaped lumen. | 4-Cinchona |
| c) Cork cell. | b) scleride. | a) acicular crystal of Ca oxalate. | 5-Ipeca |

**Physical properties of Alkaloids:**

**a)Melting Point:**

1-Atropine: 118oC.

2-Hyoscine, Hyoscyamine: 141 °C

3-Quinine: 200 - 203°C

4-Quinidine: 175-176 °C

5-Emetine: 240-250°C

6-Cephaline: 180 °C

**b)Fluorescence:**

Can be detected by TLC or by water.

quinine and quinidine give blue fluorescence

**Chemical tests of Alkaloids:**

* Mayer's reagent 🡪 Cream coloured precipitate.
* Dragendorff's reagent 🡪 orange coloured precipitate.
* Wagner’s reagent 🡪 red-brown precipitate.

Lab-6

**Evaluation of samples containing phenolic derivatives**

**Aloe**

**Definition:** The solid residue obtained by evaporating the juice flows from the cut leaves of different species of *Aloe*

**Family:** Liliaceae

**Description:**

**Condition:** Powder

**Color:** dark brown ( black)

**Odor:** characteristic

**Taste:** very bitter

**Solubility:** soluble in alcohol, insoluble in water

**Chemical tests:**

**Modified Borntrager's test:**

Powder + 5 ml dilute HCl + 5ml ferric chloride , heat in water bath for 15 min, filter while hot, then cool. Filtrate + CHCl 3 shake well, take the lower layer in test tube+ dil ammonia 🡪 rose red color in the ammonia layer (upper layer).

**Microscopial examination:**

Large prisms grouped into masses.

**Uses:**

* Cosmetic products
* In burns

**Active constituent:** Anthraquinone glycoside.

**Senna**

**Origin:**

Dried leaves of *Cassia angustifolia.*

**Family:**

Leguminosae

**Description:**

**Condition:** Powder

**Color:** Green

**Odor:** Slight

**Taste:** Characteristic

**Solubility:** Soluble in water and in dilute alcohol but insoluble in absolute alcohol.

**Microscopial examination:**

|  |  |
| --- | --- |
|  | 1-Paracytic stomata. |
|  | 2-Crystal sheath of ca.oxalate. |
|  | 3-Simple hair(non glandular). |

**Active constituents:**

Glycoside deiv. of anthraquinone e.g. sennoside A,B,C and D.

**Uses:**

Laxative.

**Chemical tests:**

Borntrager test:

Powder + 5 ml H2SO4 , boil for 2 min., filter while hot, then cool. Filtrate + extract by shaking with 5ml benzene, take the upper benzene layer in test tube+2 ml dil ammonia (shake) 🡪 rose red color in the ammonia layer (lower layer).

**Cascara**

**Origin:**

Dried bark of *Rhamnus Purshiana*

**Family:**

Rhamnaceae

**Description:**

**Condition:** Powder

**Colour:** Reddish brown

**Odour:** Slight

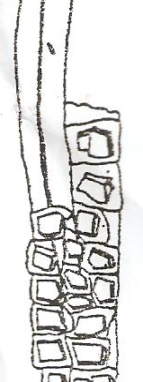
**Taste:** Characteristic

**Active constituents:**

Glycoside (cascaroside)

**Uses:**

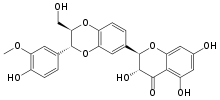
Tonic and laxative in large dose



**Microscopial examination:**

|  |  |
| --- | --- |
| 1-Phloem fiber free or with Ca.oxalate |  |
| 2-Cork cell |  |
| 3-Scleride |  |

**Silymarin**



It consists of Flavonolignans (flavonoids linked with coniferyl alcohol).

It has antihepatotoxic principles.

**Origin:**

It isolated from the fruit of Silybum marianum.

**Description:**

**Condition:** Powder

**Color:** Yellow or brown

**Odor:** Odourless

**Taste:** Bitter

**Solubility:** Soluble in water

**Uses:**

It is used in treatment of liver dysfunction.

It prevents cells against toxins.

Antioxidant and anti-inflammatory.

**Microscopical examination:**

1-Fiber

2-Starch

3-Oil gland

**Chemical tests:**

1-Spot of silymarin solution on filter paper + Fecl3➨Black spot.

2-Silymarin solution + Dil NaOH ➨ Yellow.

3- Spot of silymarin solution on filter paper + vapour of NH4OH➨Yellow spot.

**Evaluation methods of samples of fixed oils and volatile oils**

**Differences between fixed oil and volatile oil:**

|  |  |
| --- | --- |
| **Volatile oil** | **Fixed oil** |
| Volatile | Not volatile |
| Can be distilled from their natural sources | Cannot be distilled |
| Don’t consist of glyceryl esters of fatty acids | Consists of glyceryl esters of fatty acids |
| Don’t leave a spot on paper | Leave a permanent grease spot on paper |
| Don’t become rancid but it is oxidize and renisify when exposes to air | Become rancid |

**Examples of fixed oil:**

Caster oil, Olive oil and Cod liver oil.

**Physical properties:**

1-Solubility:

They are insoluble in water.

Soluble in solvents e.g. ether and chloroform.

2-Odour:

Bland.

3-Taste:

Little distinctiveness.

4-Colour:

Colourless or yellow due to presence of carotene (provitamins A).

**Physical constants:**

Including Refractive index and Specific gravity.

**Chemical constants:**

Including Acid value, Iodine value and Saponification value.

They are important for identification and judging the quality of oils.

1-Acid value:

Definition: Is the number of mg of potassium hydroxide required to neutralize the free acid in one gram of the sample.

Principle: Acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase action.

Method:

1-Mix 25ml of diethylether with 25ml alcohol and 1ml of phenolphthalein solution, and carefully neutralize with 0.1N NaOH.

2-Dissolve 5gm of Caster oil in the neutral solvent, warm to dissolve if necessary, then cool.

3-Titrate with 0.1N NaOH, shake constantly, until a pink colour with persists for 15 sec. is obtained.

Calculation:

Acid value = Titration (ml) x 0.00561 x 1000

Wt of sample

2-Iodine value:

Definition: Is the weight of iodine absorbed by 100 parts by weight of the sample.

Principle: is a measure of the degree of unsaturation, the greater the degree of unsaturation (the higher the I.V), the greater of oil liability to go rancid by oxidation.

Method: by iodinemonochloride (wij's) method.

1-Place 5 gm of Caster oil in dry, stoppered conical flask of 250ml capacity.

2-Add 10 ml CCl4 and dissolve.

3-Add 20 ml of wijs solution (iodinemonochloride), inset the stopper and allow to stand in the dark for 30 min.

4-add 15 ml of 10% KI and 100ml water, mix well.

5-Titrate the excess iodine with 0.1 N thiosulphate solution, shake after each addition, take the end point in CCl4 layer (lower layer) where it becomes colourless while the upper layer turns milky white. (titration = a ml)

6-Carry out a blank at the same time with 10 ml CCl4 (titration=b ml).

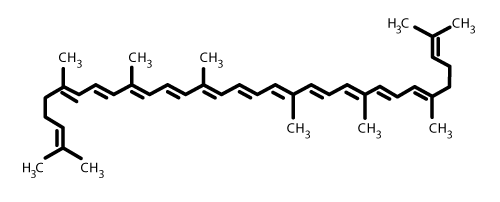
Calculation:

Iodine Value= (b-a) x 0.01269 x 100

Wt of sample (gm)

Lab-8

Isolation of lycopene from tomato paste

* 
* Lycopene C40H56 is a bright red crotenoid pigment found in tomato and other red fruits ( watermelon, grapefruit, pink guava and papaya).
* Lycopene is a tetraterpene assembled from 8 isoprene units.
* Lycopene is a very powerful antioxidant.

principle:

Tomato paste is dehydrated with methanol, then lycopene is extracted from the residue with methanol-carbon tetrachloride. The crude product is crystallized from benzene by the addition of methanol, giving lycopene of 98-99% purity.

**Procedures:**

1. Empty 2 cans of tomato paste in a beaker, and add enough methanol to cover the paste.
2. Stir the mixture with magnetic stirrer for one hour. (dehydration of tomato paste)
3. Strain using buchner funnel, and through the filtrate.
4. Transfer the paste to a bottle and shake with 350 ml of methanol and 350 ml of CCl4 (divided in three times) and strain using buchner funnel.
5. Combine the filtrate.
6. Transfer to a separating funnel and pass the lower methanolic layer over anhydrous Na2SO4.
7. Evaporate all the methanol to obtain an oily residue
8. Crystalline the lycopene using benzene.
9. Collect the crystals in a vial and weigh.

Lab-9

**Isolation of pectin from grape fruits**

Pectin refers to a group of diverse and complex polysaccharides found in the primary cell wall and intercellular space (middle lamella) of plant cells. Pectin is mostly composed of a sugar residue called D-galacturonic acid. The dominant polysaccharides in pectin are homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, and xylogalacturonan. Pectin is a [carbohydrate](http://www.dietaryfiberfood.com/sugar-in-food/cabohydrate-food-chart.php).

**Sources of pectin:**

Pectin is higher in legumes and citrus fruits than cereals. [Apple](http://www.dietaryfiberfood.com/apple-fruit-benefits.php), grapefruit, orange and apricot. Generally, 60 – 70 % of the dietary fiber in citrus fruits is pectin. Other sources of pectin include banana, beets, cabbage, carrots.

**Uses:**

Pectin is classified as suspending agent and as ingredient in many antidiarrheal formulations.

**Principle:**

Pectin in fruit are found insoluble form known as protopectin, convert to the soluble form by heating the fruit by dilute acid. Pectin can ppt. by alcohol.

**Procedure:**

1- Carefully peel the yellow layer from a rind of a citrus fruit and discard.

2- Remove the white inner rind and cut into small pieces.

3- Transfer the material to a beaker after collecting in cotton gauze.

4- Cover with water and boil for 1 hour, maintaining the volume of liquid by frequent addition of water.

5- Filter, cool the filtrate and add twice its volume of alcohol.

6-Filter the precipitated pectin and dry at room temperature.