S.A.RAJA PHARMACY COLLEGE

VADAKKANGULAM 627 116

IV SEMESTER (II-B.PHARM)

PHARMACOGNOSY AND PHYTOCHEMISTRY -I

PRACTICAL LAB MANUAL

EX No1 Chemical test: for Tragacanth.

Aim: To identify the chemical characters of given sample.

Background

Unorganized drugs, as the name suggests, are drugs that show no definite cellular structure. These are derived from plant, animal or mineral sources by some process of extraction and followed by purification if necessary. Unorganized drugs are fairly homogenous and may be solids, semisolids or liquids. These may be differentiated by observing the solubility in alcohol and then applying other physical and chemical standards. TragacanthTragacanth is the dried gummy exudation from the stem of Astragalusgummifer, Labillardiere and other species of Astragalus (Fam. Leguminosae). It occurs in flat or curved ribbon shaped flakes. Odourless,n almost tasteless, white or pale yellowish-white, somewhat translucent, horny; fracture short.

Chemicals Required :

Hydrochloric acid, Sodium hydroxide solution, Fehling's solution, Barium chloride solution, Lead acetate, Ruthenium red, Iodine Caustic potash.

PROCEDURE

1). To 4 ml of 0.5% w/v solution, add 0.5 ml of hydrochloric acid and heat for 30 minutes on a water bath. Divide the liquid into two parts. (a). To one part, add 1.5 ml of sodium hydroxide solution and Fehling's solution, warm on water bath: red precipitate is produced. (b). To the second part, add barium chloride solution (10%): No precipitate is obtained (distinction from agar)

2). To a 0.5% w/v solution of the gum, add 20% w/v solution of lead acetate: A voluminous flocculent precipitate is obtained (distinction from acacia)

3). Mount a small quantity of powder in ruthenium red and examine microscopically: Particles do not acquire pink colour (distinction from Indian tragacanth)

4). To 0.1 g of powder, add N/50 Iodine: The mixture acquires an olive green colour (distinction from acacia and agar).

5). Powder is warmed with 5% aqueous caustic potash: Canary yellow colour will obtain. Indian tragacanth .It is obtained from Sterculiaurens Roxburgh; (Fam: Sterculiaceae). It is insoluble in alkali. It has acetous (acetic acid like) odour and starch is absent. It gives brownish colour when boiled with aqueous KOH. It is stained pink by solution of Ruthenium red.

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as Tragacanth.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, Vallabh Prakashan. Delhi; 1994: 98

EX No 2 Chemical test: for Acacia

Aim: To identify the chemical characters of given sample.

Constituents:

Gum Arabic consists almost entirely of glycosidal acid named Arabic acid, combined with potassium, magnesium and calcium. By hydrolysis Arabic acid yields 1 molecule of l-rhamnose, 2 molecules of D – galactose and 3 molecules of l– arabinose and an aldobionic acid. It also contains diastase and an oxidase enzyme.

PROCEDURE :

1) Dissolve about 0.25 gm of the coarsely powdered drug in 5 ml of distilled water by shaking in the cold. Add 0.5 ml of hydrogen peroxide and 0.5 ml of benzidinesolution, shake and allow to stand for few minutes; a deep blue color or greenish blue color is formed due to the prescence of oxidase enzyme.

2) A 10% aqueous solution of acacia fails to produce any precipitate with dilute solution of lead acetate (a clear distinction from Agar and Tragacanth); it does not give any colour change with Iodine solution (a marked distinction from starch and dextrin); and it never produces a bluish-black colour with FeCl3 solution (an apparent distinction from tannins).

3) Hydrolysis of an aqueous solution of acacia with dilute HCl yields reducing sugars whose presence are ascertained by boiling with Fehling's solution to give a brick-red precipitate of cuprous oxide

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as Acacia.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98

EX No 3 Chemical test: for Agar

Aim: To identify the chemical characters of given sample.

SI.No.	Test	Observation	Inference
1.	Boil 1gm of agar with 10ml of water until solution is affected, cool to room temperature	A slip jet is formed. (Jelly like mass is formed)	Agar
2.	0.2% solution of agar, + aqueous solution of tannic acid	No Precipitate is formed	Distinction from gelatin
3.	Warm little sample in alcoholic solution of pot. Hydroxide	Canary yellow colour is produced	Agar present
4.	Mount a small quantity of powder in the solution of ruthenium red and examine microscopically	Particles acquire red or pink colour	Presence of mucilage in agar.
5.	Add 1 drop of N/10 solution of iodine to 10ml of decoction of agar. Rapidly cool under tap water to room temperature.	Crimson or Pale yellow colour is produced	Agar is present
6.	Add 0.5ml of conc. HCl to 4ml of 0.5% solution of agar. Heat it on water bath for 30minutes, cool at room temperature and divide into two portions. a) Add 3ml of 10% NaOH solution and Fehling's solution A and B in equal quantities and warm over water bath. b) Add 10% of barium chloride solution.	Red ppt of cuprous oxide is obtained Slight white ppt of barium sulphate is obtained	Reducing sugars are present
7.	Incinerate agar to ash, add a drop of con.HCl observe under microscope	Fragments of diatoms	Agar is present

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as Agar.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98.

EX No 4 Chemical test: for Gelatin

Aim: To identify the chemical characters of given sample.

Background

Gelatin is occurs in thin sheets, strips or as granular powder. High grade gelatin light yellow, semi crystalline substance. It is Odourless and tasteless. In cold water it swells up and slowly dissolves on warming to form viscous solution.

Chemical Tests:

1) When gelatin is heated with soda lime in dry test tube, ammonia is evolved due to the presence of nitrogenous compound in gelatin.

2) Gelatin solution is added Million's reagent to give a white ppt, which turns red on heating.

3) Gelatin gives buff white ppt with tannic acid solution.

4) Biuret test to 3 ml of test solution of gelatin. NaOH (1ml of 5%) is added whereby white to whitish buff colouredppt is formed which does not dissolve on heating.

5) Yellow ppt. is formed on adding picric acid to solution of gelatin.

6) It gives yellow ppt. with trinitrophenol in aqueous solution.

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as Gelatin.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98

EX No 5 Chemical test: forstarch

Background

Amylose forms a colloidal dispersion in hot water whereas amylopectin is completely insoluble. The structure of amylose consists of long polymer chains of glucose unitsconnected by an alpha acetal linkage. Starch - Amylose shows a very small portion of an amylose chain. All of the monomer units are alpha -D-glucose, and all the alphaacetal links connect C #1 of one glucose and to C #4 of the next glucose. As a result of the bond angles in the αacetal linkage, amylose actually forms a spiral much like acoiled spring. See the graphic below, which show four views in turning from a the side to an end view

Chemical Test for Starch or Iodine

Amylose in starch is responsible for the formation of a deep blue color in the presence of iodine. The iodine molecule slips inside of the amylose coil. Iodine - KI Reagent: Iodine is not very soluble in water; therefore the iodine reagent is made by dissolving iodine in water in the presence of potassium iodide. This makes a linear triiodide ion complex with is soluble that slips into the coil of the starch causing an intense blue-black color.

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as starch.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98

EX No 6 Chemical test: for Honey

SI.No.	Test	Observation	Inference
1.	**Fiehe's test** Take about 3ml of honey + 2ml of ether and shake thoroughly and allow the 2 layers to separate and evaporate to dryness. The upper etherial layer is separated and put in a china dish and evaporate, to the residue add 1% resorcinol and HCl.	Transient pink colour Permanent red colour	Pur honey Adulterated honey(Invert sugar)
2.	**Molisch's Test** Honey is treated with alpha Napthol and concentrated sulphuric acid	Purple colour	Presence of carbohydrate
3.	**Reducing Sugar Test** Heat honey to this add a drop of mixture of Fehling's solution A & B	Brick red colour of cuprous oxide	Presence of monosaccharide

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as Honey.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98

EX No 7 Chemical test: for Castor oil

SI.No	Test	Observation	Inference
1.	Add 5ml of light petroleum ether (40^{o} -60^{o}) to 10ml of castor oil	A clear solution results and on increasing the petroleum ether about 15ml, turbid mixture will be obtained	Castor oil present
2.	Oil + equal volume of alcohol and cool to 0^{o}C for 3hrs	A clear liquid is obtained	Castor oil is present.

CONCLUSION From the above morphological characters and chemical tests the given crude drug is identified as castor oil.

REFERENCES 1.Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 98

EX No 8

DETERMINATION OF STOMATAL NUMBER

BACKGROUND

Stoma (plural-stomata) is a minute epidermal opening covered by two kidney shaped guard cells in dicot leaves. These guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomata perform the functions of gaseous exchange and transpiration in plants. The nature of the stomata, as well as, the stomatal index and stomatal number are important diagnostic characteristics of dicot leaves. Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf. The actual number of stomata per sq mm may vary for the leaves of the same plant grown in different environment or under different climatic conditions. It is, however shown that the ratio of the number of stomata to the total number of epidermal cells in a given area of epidermis is fairly constant for any age of the plant and under different climatic conditions. Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following equation:

Stomatal Index = S x 100/E+S

Where, S= Number of stomata per unit area E= Number of epidermal cells in the same unit area

Whilst stomatal number varies considerably with the age of the leaf and due to changes in environmental conditions, stomatal index is relatively constant and therefore, of diagnostic significance for a given species. It is employed for the differentiation of allied or closely related to species of same genus in air dried, as well as fresh conditions

REQUIREMENTS Compound microscope Camera lucida Drawing board Micro slides Cover glasses Forceps Spirit lamp Small watch glass Blade Cello tape, drawing sheet Dark coloured pencil with sharp lead Chloral hydrate solution

PROCEDURE

1. Preparation of lamina

Take a mature leaf. If the leaf is small, the whole leaf may be taken and if the leaf is large,cut 5 mm square pieces from themiddle portion between the lamina and midrib.

Fresh leaf

1) Sometimes the epidermis can be easily peeled off in thick leaves by breaking it into pieces by sheering action. Separate the epidermis and treat with chloral hydrate.
2) Cut a number of 5mm pieces from the middle portion between the lamina and midrib.

3) Boil with chloral hydrate in a test tube placed in a water bath. The epidermis separates out. Carefully place the
4) epidermis on a slide with the help of a brush along with 1-2 drops of chloral hydrate; cool and then place a cover glass.
5) OR
6) Prepare an imprint of the epidermis: Take a little piece of gelatin gel (50%) with the help of a needle. Smear it on a hot slide, place a fresh leaf and slightly press the leaf. Invert the slide and cool it under a water tap till the gel is solidified.
7) Then the leaf is removed. This leaves an imprint of the stomata and epidermal cells on the gel.
8) Trace the epidermal cells and stomata with the help of camera lucida.

Dry leaf

1) Heat the leaf with chloral hydrate in a test tube on a water bath for 30 min.
2) Cut the leaf into two pieces, observe under the microscope to see whether the stomata are present on both surfaces or one.
3) Place the cleared leaf with the veins facing down. Then the upper epidermis will be visible.
4) Place the other half with veins facing upwards. Then the lower epidermis will be visible.
5) Add two drops of glycerin and place a cover glass.
6) Label the slides as “upper” and “lower” and trace the epidermal cells and stomata.

If the leaf is too thick and dark, separate the epidermis are given below.

1) Clear the leaf with chloral hydrate as given in step no.1. cut the leaf into two halves.
2) Place one half with the upper surface facing downwards.
3) Carefully scrape off the upper tissue, with the edge of a razor blade, without disturbing upper epidermis. Clean it with a
4) brush dipped in chloral hydrate solution.
5) The layer of cells remaining on the is the upper epidermis. Turn the layer upside to trace the cells.
6) Repeat the procedure with the second half, this time placing the lower surface facing downwards, proceed as given in

step no. 3. and 4.

6. Usually herbs and small shrubs have stomata on both surfaces. In tree species, stomata are present on the lower surface.

More stomata are present on the lower surface in dorsiventral leaf, almost the same number in isobilateral leaf.

2. Tracing of cells

In this experiment the number has to be determined per square millimeter.

1) Adjust the drawing board, if swift camera-lucida is used. It is not necessary to adjust angle with Abbe's camera lucida.
2) With the help of stage micrometer, draw a line of 1mm using 10×10 magnification on a drawing sheet and draw a 10 cm
3) square on that line (1 mm =10 cm) if magnification is correct.
4) Replace the stage micrometer with a prepared slide of the leaf as given for stomatal index.
5) With the help of camera lucida mark the number of stomata in the square.
6) Count the stomata. That gives the number of stomata per sq. mm.
7) Take 25 reading and calculate the average.
8) Note the side, for which the stomatal number is determined. Direct counting of stomata can also be done by a squared eyepiece micrometer, if the drawings are not required

CONCLUSION

Stomatal number and stomatal index of supplied leaf is _____

REFERENCES

1. Kokate CK, Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 117-118.

2. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 208-09.

EX No 9 **DETERMINATION OF STOMATAL INDEX**

BACKGROUND

Stoma (plural-stomata) is a minute epidermal opening covered by two kidneys shaped guard cells in dicot leaves. These guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomata perform the functions of gaseous exchange and transpiration in plants. The nature of the stomata, as well as, the stomatal index and stomatal number are important diagnostic characteristics of dicot leaves. Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf. The actual number of stomata per sq mm may vary for the leaves of the same plant grown in different environment or under different climatic conditions. It is, however shown that the ratio of the number of stomata to the total number of epidermal cells in a given area of epidermis is fairly constant for any age of the plant and under different climatic conditions. Stomatal index is the percentage which the number of stomata form to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following equation:

$$\text{Stomatal Index} = S \times 100/E+S$$

Where, S= Number of stomata per unit area E= Number of epidermal cells in the same unit area. Whilst stomatal number varies considerably with the age of the leaf and due to changes in environmental conditions, stomatal index is relatively constant and therefore, of diagnostic significance for a given species

REQUIREMENTS

Compound microscope Camera lucida Drawing board Micro slides Cover glasses Forceps Spirit lamp Small watch glass Blade Cello tape Drawing sheet, Dark coloured pencil with sharp lead Chloral hydrate solution.

PROCEDURE

1. Preparation of lamina Take a mature leaf. If the leaf is small, the whole leaf may be taken and if the leaf is large cut 5 mm square pieces from the middle portion between the lamina and midrib. Fresh leaf 1. Sometimes the epidermis can be easily peeled off in thick leaves by breaking it into pieces by sheering action. Separate the epidermis and treat with chloral hydrate.

2. Cut a number of 5mm pieces from the middle portion between the lamina and midrib.

3. Boil with chloral hydrate in a test tube placed in a water bath. The epidermis separates out. Carefully place the epidermis on a slide with the help of a brush along with 1-2 drops of chloral hydrate; cool and then place a cover glass. OR

4. Prepare an imprint of the epidermis: Take a little piece of gelatin gel (50%) with the help of a needle. Smear it on a hot slide, place a fresh leaf and slightly press the leaf. Invert the slide and

cool it under a water tap till the gel is solidified. Then the leaf is removed. This leaves an imprint of the stomata and epidermal cells on the gel.

5. Trace the epidermal cells and stomata with the help of camera lucida.

Dry leaf

1. Heat the leaf with chloral hydrate in a test tube on a water bath for 30 min.

2. Cut the leaf into two pieces, observe under the microscope to see whether the stomata are present on both surfaces or one.

3. Place the cleared leaf with the veins facing down. Then the upper epidermis will be visible.

4. Place the other half with veins facing upwards. Then the lower epidermis will be visible.

5. Add two drops of glycerin and place a cover glass.

6. Label the slides as “upper” and “lower” and trace the epidermal cells and stomata.

If the leaf is too thick and dark, separate the epidermis are given below. 1. Clear the leaf with chloral hydrate as given in step no.

1. cut the leaf into two halves.

2. Place one half with the upper surface facing downwards.

3. Carefully scrape off the upper tissue, with the edge of a razor blade, without disturbing upper epidermis. Clean it with a brush dipped in chloral hydrate solution.

4. The layer of cells remaining on the upper epidermis. Turn the layer upside to trace the cells.

5. Repeat the procedure with the second half, this time placing the lower surface facing downwards, proceed as given in step no. 3. and 4.

6. Usually herbs and small shrubs have stomata on both surfaces. In tree species, stomata are present on the lower surface. More stomata are present on the lower surface in dorsiventral leaf, almost the same number in isobilateral leaf.

Tracing of cells

1. Draw a square of about 8-10 cm square on a drawing sheet or any unit area.

2. Place the prepared slide on the stage of the microscope.

3. Focus epidermal cells and the stomata first with 10×10 and later focus with 10×40 or 10×20.

4. With the help of camera lucida, trace the stomata and the epidermal cells in the square.

5. Trace epidermal cells and the stomata outside the square to completion on two adjacent sides of the square, for counting purpose.

6. Number the complete epidermal cells and the stomata within the square.

7. Then continue numbering the cells that are more than half on two adjacent sides.

Calculation ----------Stomatal index = no. of stomata x 100 No. of stomata + epidermal cells

REFERENCES

1. Kokate CK, Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 117-118.

2. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 208-09

EX No 10

DETERMINATION OF VEIN-ISLET AND VEIN TERMINATION NUMBER

Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per mm of leaf surface. A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The average number of vein islet from the four adjoining square, to get the value for one square mm was calculated. The number of veinlet termination present within the square was counted and the average number of veinlet termination number from the four adjoining square to get the value for 1 square mm was found known as vein termination number.

EX No 11

DETERMINATION OF PALISADE RATIO

A piece of the leaf was boiled in chloral hydrate and was placed under microscope. Camera lucida and drawing board were arranged and the outline of four cells of the epidermis was traced using 4 mm objective. Then, palisade layer was focused down and sufficient cells for covering the tracing of the epidermal cells were traced off. The outline of those palisade cells which were intersected by the epidermal walls was completed. The palisade cells under the four epidermal cells (including cells which are more than half and excluding cells which are less than half within the area of epidermal cells) were counted. The determination for five groups of four epidermal cells from different part of the leaf was repeated. The average number of cells beneath epidermal cells was calculated known as palisade ratio.

EX No 12

DETERMINATION OF SIZE OF STARCH GRAINS

BACKGROUND

Measurement of crystals using stage and eyepiece micrometers.

REQUIREMENTS

Microscope with mechanical stage

Eyepiece and stage micrometer

Calibration of eyepiece micrometer with the help of stage micrometer:

1. **Stage micrometer**

1. Take the micrometer slide in hand and feel the sides of the slide.

2. The correct sides shows a slight elevation on one side.

3. Place it on the stage of the microscope. View the scale through the eyepiece at 10 x 10 magnification to find theposition of the scale on the micrometer.

4. To locate the scale, start viewing from the edge of the cover glass containing the engraved micrometer scale andthen move to the centre and locate the scale on the equatorial plane.

2. **Eyepiece micrometer**

1. Remove the eyepiece and unscrew the part A

2. Place the eyepiece micrometer at level B of the eye piece where there is a hinge or diaphragm (it is the support onwhich the eyepiece micrometer rests).

3. Screw part A back and see whether the numbering on the eyepiece micrometer is in correct position, i,e zero tohundred. If numbers are reverse, remove the scale and place it in a correct manner.

4. The eye-piece can be rotated to make adjustments.

5. Do not introduce the eyepiece micrometer into the draw tube.

3. Calibration of eyepiece micrometer

1. View through the eyepiece with the required optical combinations.

2. Adjust the scales of the two micrometers such that both the scales are superimposed. Rotate the eyepiece to place

the scales in a parallel position or remove the stage micrometer till the lines coincide with eyepiece scale.

3. Move the stage micrometer such that the “0” readings of both the micrometer scales coincide or one of the largerdivision of the stage micrometer coincides with one of the lines of the eyepiece micrometer scale. Note the initialreadings.

4. Carefully scan the scales to see, which of the two scale readings exactly coincide on the right side. Note the finalreadings and calculate the factor for 1 division of the eyepiece micrometer.

Now the microscope is ready for taking measurements

Compound microscope

Eyepiece micrometer

Stage micrometer

Glycerin water

PROCEDURE

Calibrate eyepiece micrometer by using stage micrometer and calculate the factor. Mount a little quantity of powdered samplein glycerin water and measure the length and breadth of 25 calcium oxalate crystals. Multiply the values by the factor for thenext dimensions of the crystals.

Calculate the average value and give the range for the dimensions.

In case of cascara bark the calcium oxalate crystals range from 10-25-45 µm, while in case of frangula bark, they measure upto 15 µm.

CONCLUSION

Size of calcium oxalate crystals of the given sample is _____

REFERENCES

1. Kokate CK, Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 112-114.

2. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 203-204.

EX No 13

DETERMINATION OF SIZE OF CALCIUM OXALATE CRYSTALS

BACKGROUND

Measurement of crystals using stage and eyepiece micrometers.

REQUIREMENTS

Microscope with mechanical stage

Eyepiece and stage micrometer

Calibration of eyepiece micrometer with the help of stage micrometer:

1. Stage micrometer

1. Take the micrometer slide in hand and feel the sides of the slide.

2. The correct sides shows a slight elevation on one side.

3. Place it on the stage of the microscope. View the scale through the eyepiece at 10 x 10 magnifications to find theposition of the scale on the micrometer.

4. To locate the scale, start viewing from the edge of the cover glass containing the engraved micrometer scale andthen move to the centre and locate the scale on the equatorial plane.

2. Eyepiece micrometer

1. Remove the eyepiece and unscrew the part A

2. Place the eyepiece micrometer at level B of the eye piece where there is a hinge or diaphragm (it is the support onwhich the eyepiece micrometer rests).

3. Screw part A back and see whether the numbering on the eyepiece micrometer is in correct position, i,e zero tohundred. If numbers are reverse, remove the scale and place it in a correct manner.

4. The eye-piece can be rotated to make adjustments.

5. Do not introduce the eyepiece micrometer into the draw tube.

3. Calibration of eyepiece micrometer

1. View through the eyepiece with the required optical combinations.

2. Adjust the scales of the two micrometers such that both the scales are superimposed. Rotate the eyepiece to placethe scales in a parallel position or remove the stage micrometer till the lines coincide with eyepiece scale.

3. Move the stage micrometer such that the “0” readings of both the micrometer scales coincide or one of the largerdivision of the stage micrometer coincides with one of the lines of the eyepiece micrometer scale. Note the initialreadings.

4. Carefully scan the scales to see, which of the two scale readings exactly coincide on the right side. Note the finalreadings and calculate the factor for 1 division of the eyepiece micrometer.

Now the microscope is ready for taking measurements

Compound microscope

Eyepiece micrometer

Stage micrometer

Glycerin water

PROCEDURE

Calibrate eyepiece micrometer by using stage micrometer and calculate the factor. Mount a little quantity of powdered samplein glycerin water and measure the length and breadth of 25 calcium oxalate crystals. Multiply the values by the factor for thenext dimensions of the crystals.

Calculate the average value and give the range for the dimensions.

In case of cascara bark the calcium oxalate crystals range from 10-25-45 µm, while in case of frangula bark, they measure upto 15 µm.

CONCLUSION

Size of calcium oxalate crystals of the given sample is _____

REFERENCES

1. Kokate CK, Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 112-114.

2. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 203-204.

EX No 14

DETERMINATION OF FIBER LENGTH AND WIDTH

BACKGROUND

Fiber is composed of plants which resist human digestive enzymes, a definitionthat includes lignin and polysaccharides, are of twotypes water soluble and insoluble. Fibers are natural and synthetic.

Aim To determine length and width of fibers of Chinchona Bark Powder

REQUIREMENTS

Microscope with mechanical stage

Eyepiece and stage micrometer

Glycerin water

Phloroglucinol

Concentrated hydrochloric acid

Chloral hydrate solution

PROCEDURE

Calibration of eyepiece micrometer with the help of stage micrometer

1. Stage micrometer

2. Take the micrometer slide in hand and feel the sides of the slide.

3. The correct sides shows a slight elevation on one side.

4. Place it on the stage of the microscope. View the scale through the eyepiece at 10 x 10 magnification to find theposition of the scale on the micrometer.

5. To locate the scale, start viewing from the edge of the cover glass containing the engraved micrometer scale and thenmove to the centre and locate the scale on the equatorial plane.

6. Eyepiece micrometer

7. Remove the eyepiece and unscrew the part A

8. Place the eyepiece micrometer at level B of the eye piece where there is a hinge or diaphragm (it is the support onwhich the eyepiece micrometer rests).

9. Screw part A back and see whether the numbering on the eyepiece micrometer is in correct position, i,e zero tohundred. If numbers are reverse, remove the scale and place it in a correct manner.

10. The eye-piece can be rotated to make adjustments.

11. Do not introduce the eyepiece micrometer into the draw tube.

12. Calibration of eyepiece micrometer

13. View through the eyepiece with the required optical combinations.

14. Adjust the scales of the two micrometers such that both the scales are superimposed. Rotate the eyepiece to place thescales in a parallel position or remove the stage micrometer till the lines coincide with eyepiece scale.
15. Move the stage micrometer such that the "0" readings of both the micrometer scales coincide or one of the largerdivision of the stage micrometer coincides with one of the lines of the eyepiece micrometer scale. Note the initialreadings.

16. Carefully scan the scales to see, which of the two scale readings exactly coincide on the right side. Note the finalreadings and calculate the factor for 1 division of the eyepiece micrometer.

Now the microscope is ready for taking measurements.

Using stage micrometer calibrate the eyepiece micrometer. Calculate the factor (average distance between two lines inmicrons). Take a little quantity of powder drug (Ceylon cinnamon, Cassia bark or Cinchona bark) in a test tube and boil withclearing agent, chloral hydrate solution. Transfer the cleared powder in a watch glass. Stain the lignified fibers with thestaining reagent (phloroglucinol and concentrated hydrochloric acid). Mount this treated powder in glycerin water andobserve the slide under low power. (Power should be thinly, uniformly scattered, without overlapping of particles). Focus astained fiber (intact fiber). By rotating the scale of eyepiece micrometer, note the numbers of divisions of the eye piecemicrometer covered by the length of the fiber. Again rotate the eyepiece micrometer without disturbing the slide and find thenumbers of division of the eyepiece micrometer covered by the width of the same fiber. Similarly calculate the length andwidth of about 25 fibers, and write the readings in two separate columns.

Multiply each value by the factor calculated in the first step to get the value in microns. Then calculate the average value andwrite the range for the length and the width fiber.

CONCLUSION

Length and width of the fibre present in the given bark powder _____

REFERENCES

1. Khandelwal KR. Practical Pharmacognosy: Techniques and experiments, 9 edition, NiraliPrakashan. Pune; 2002: 159.

EX No 15

DETERMINATION OF NUMBER OF STARCH GRAINS BY LYCOPODIUM SPORE METHOD

BACKGROUND

Lycopodium spores are obtained from club moss, LycopodiumclavatumLinn., belonging to family Lycopodiaceae. Thespores are yellow in colour, spheroidol, tetrahedral in shape with reticulate surface. They have uniform average diameter of 25microns. One milligram contains average 94000 spores. They have uniform moisture content, hence the weight remains thesame. This is the reason, why these spores are used to evaluate powdered drugs by comparison. The spores are also resistantto pressure.

REQUIREMENTS

Balance

Watch glass

Small flexible spatula

Microscope with mechanical stage or a counting square

Suspending agent: Fixed oil or suspending agent; glycerine: tragacanth mucilage: water (2:1:2). This keeps the spores andparticles in a suspension. Dilution of the suspension should give about 10 to 20 spores in a field.

PROCEDURE

Determine the loss on drying for the powder at 105 C. Mix a weighed amount of air-dry powder of the drug and a weighedamount of lycopodium spores in a small watch glass (100 mg drug and 50 mg lycopodium spores). Mix with a small flexiblespatula. Add oil or suspending agent. Mix for 10 min till a smooth paste is obtained.

Transfer the suspension to a small glass tube by draining with the help of a glass rod. Add more suspending agent, washingdown the mixture into the tube. (about 4 ml of the suspending agent is required for 50 mg of lycopodium spores). This shouldgive about 10 to 20 spores when viewed under 4 mm objective, when a drop of the mixture is mounted under a cover glass.

Slowly oscillate the glass tube between the two palms without any air bubbles, until the suspension is uniform. Take a glasstube with internal diameter of about 2-3 mm and place one drop each on two sides, spread the suspension on the slide lessthan the area of the cover slip. Apply a cover slip and leave the slide on an even surface to settle.

Select 25 fields and count the spores and particles in these fields using 10×40 magnification.

Make a similar suspension as above and count particles in 25 fields on two sides.

Take average of 4 readings.

Calculate the percentage of foreign organic matter from the formula given below:

Percentage of foreign organic matter = 94000 x 100 x n x w / s x m x p

m= weight in mg of the sample, calculated on sample dried at 105 C.

w= weight in mg of the lycopodium spores

n= number of particles in 25 fields

p= number of particles in per mg of the pure foreign matter dried at 105 C

94,000= number of spores in one mg of lycopodium

Calculations

1 mg of lycopodium powder contains spores = 94000

W mg of lycopodium powder powder contains 94000 x w number of spores.

s number of spores in ten fields mix with n number of starch grains in ten fields.

94000 x w number of spores mix with = 94000 x n x w / s (number of starch grain)

1 mg of pure sample of foreign organic matter (F.O.M) contains p number of starch grains

Weight of ginger in the mixture= 94000 x n x w/ s x p (mg of ginger)

m (mg) of mixture contain = 94000 x n x w/ s x p

1 mg of mixture contains = 94000 x n x w/ s x p x m

Percentage of F.O.M = 94000 x n x w x 100/ s x p x m

CONCLUSION

Number of starch grains in the given sample is _____

REFERENCES

1. Joshi S, Aeri V, Practical Pharmacognosy. 1 edition, Frank Bros. & Co. New Delhi; 2009: 211-212.

EX No 16

DETERMINATION OF ASH VALUE

BACKGROUND

Ash values are helpful to determine the quality as well as purity of a crude drug, especially when the drug is present inpowdered form. The object of ashing crude drugs is to remove the traces of organic matter which may be interferes in ananalytical determination. On incineration, the crude drugs normally produce ash which is usually consisting of carbonates,phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reveals the care takenduring its preparation. A higher limit of acid- insoluble ash is incorporated especially in cases where silica may be present orwhen the calcium oxalate content of the drug is very high. Some researchers suggested mixing of acids like sulphuric acidwith the powdered crude drug before ashing and making the ash sulphated which is normally less fusible than ordinary ash.

The present study is designed to determine the ash value of the supplied sample.

REQUIREMENTS

Chemicals: Dilute hydrochloric acid

Apparatus: Silica crucible

Dessicator

Ash less filter paper

Sample: Powdered liquorice

PROCEDURE

Total ash determination

Weigh accurately about 3 gm of the powdered drug in silica crucible. Incinerate the powdered drug by increasing the heatgradually until the sample was free from carbon and cool it keep it in a desiccators. Weigh the ash and calculate thepercentage of total ash in contrast to the air dried sample.

Determination of acid-insoluble ash

Boil the total ash obtained as the above procedure for 5 minutes and mix 25 ml of dilute hydrochloric acid. Filter and collectthe insoluble matter on a ashless filter paper, after that wash the filter paper with hot water, ignite in tared crucible, cool andkeep in desiccator. Weigh the obtained residue and calculate acid-insoluble ash of the crude drug (liquorice) with reference to

the air dried drug. The unpeeled variety of liquorice root must contain not more than 10% of total ash and 2.5% of acidinsolubleash.

CONCLUSION

Ash value of supplied sample is _____.

REFERENCES

1. Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994.

EX No 17

DETERMINATION OF WATER-SOLUBLE EXTRACTIVE VALUE OFGINGER

BACKGROUND

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractivevalues by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectlyprocessed drugs.

Crude drugs contain a number of constituents and these have a selective solubility in different solvents. Water, alcohol, alcohol/water mixtures, generally 45%, 60%, 90% ethanol, ether are used as solvents to prepare ethanol soluble extractive,water soluble extractive (chloroform water), ether soluble extractive, etc.

Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification andsemi-quantitative analysis from these extracts.

Ginger contains some water soluble constituents. If the ginger is exhausted with water or soaked in water for a long timewhile cleaning, the water soluble extractive will have a lower value than the official limit. However, Ginger exhausted withchloroform water passes this test.

REQUIREMENTS

Water soluble extractive not less than 10%

250 ml conical flask with stopper

Chloroform Water

Shallow flat-bottomed dish

Water bath

PROCEDURE

Weigh accurately about 5 g of coarsely powdered drug into a 250 ml conical flask with stopper. Add 100 ml of chloroformwater. Shake the flask frequently during first 6 hr. Keep it aside without disturbing for 18 hr. and then filter. Pipette out 25 mlof the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath. Then dry the residue at 105^{o}C to a constant weight. Calculate the percentage of water-soluble extractive.

% of water soluble extractive = weight of residue/weight of the drug x 100.It is expressed as percent w/w of the air-dried drug.

CONCLUSION

Water-soluble extractive value of Ginger is ____.

REFERENCES

1. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 255

EX No 18

DETERMINATION OF ALCOHOL-SOLUBLE EXTRACTIVE VALUE OFGINGER

BACKGROUND

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractivevalues by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectlyprocessed drugs.

Crude drugs contain a number of constituents and these have a selective solubility in different solvents. Water, alcohol,alcohol/water mixtures, generally 45%, 60%, 90% ethanol, ether are used as solvents to prepare ethanol soluble extractive,water soluble extractive (chloroform water), ether soluble extractive, etc.

Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification andsemi-quantitative analysis from these extracts.

Ginger contains about 1-3 % volatile oil and 5-8 % resins, which are soluble in alcohol.

REQUIREMENTS

Alcohol soluble extractive of ginger (should not be less than 4.5 %. Lower values indicate exhausted ginger with alcohol.

Ginger exhausted with alcohol passes this test).

250 ml conical flask with stopper

Alcohol (90%)

Shallow flat-bottomed dish

Water bath

PROCEDURE

1. Weigh accurately about 5 g of coarsely powdered drug into a 250 ml conical flask with stopper.

2. Add 100 ml of alcohol (90%).

3. Shake the flask frequently during first 6 hr.

4. Keep it aside without disturbing for 18 hr. and then filter.

5. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath.

6. Then dry the residue at 105 C to a constant weight.

7. Calculate the percentage of alcohol-soluble extractive.

8. % of alcohol soluble extractive = weight of residue/weight of the drug x 100

It is expressed as percent w/w of the air-dried drug.

CONCLUSION

Water-soluble extractive value of Ginger is ____.

REFERENCES

1. Joshi S, Aeri V. Practical Pharmacognosy, 1 edition, Frank Bros. & Co. New Delhi; 2009: 255

EX No 19

DETERMINATION OF MOISTURE CONTENT OF CRUDE DRUGS

BACKGROUND

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperaturemoisture will lead to the activation of enzymes and gives suitable conditions to the proliferation of living organism. As mostvegetable drugs contain all the essential food requirements for mould, insects and mites, deterioration can be very rapid, onceinfestation has taken place. Various methods for moisture determination are loss on drying, separation and measurement ofmoisture, chemical methods, electrometric methods, and spectroscopic methods as per IP.

REQUIREMENTS

Moisture content apparatus

Desiccators

Weighing Machine

PROCEDURE

10 gm of powder was weighed and placed it in a moisture content apparatus. Temperature was adjusted to 100-110 C tillweight get constant and collected in desiccator and weighed. The loss of weight was regarded as a measure of moisturecontent as per IP.

CONCLUSION

Moisture content of the given sample is _____

REFERENCES

1. Quality control methods for medicinal plants material by W.H.O. Guidelines: 45

EX No 20

DETERMINATION OF SWELLING AND FOAMING INDEX

BACKGROUND

Many herbal drugs are of specificfor the therapeutic or pharmaceutical utility because of their swelling properties − especially gumsand drugs those are containing an appreciable amount of constituents like mucilage, pectin or hemicelluloses. The swelling index is defined as the volume in ml taken up by the swelling of 1 g of herbal material under specied conditions. Its determination is based on the addition of water or a swelling agent as specied in the test procedure for each individual herbal material (either whole, cut or

Pulverized). Using a measuring cylinder with glass-stopper, the material must be shaken repeatedly for 1 hour and then allowed themeasuring cylinder to stand for a required period of time. The volume of the mixture (in ml) is then read. The mixing of whole herbalmaterial with the swelling agent is easy to achieve, but cut or pulverized materials requires vigorous shaking at speciedinterval oftime to ensure even distribution of the material in the swelling agent.

Aim: The main objective of present experiment is to measure the swelling factor of the given sample i.eisapgol seeds.

REQUIREMENTS

Apparatus: Stoppered measuring cylinder – 25 ml

Conical flask – 500 ml

Volumetric flask

Stopper test tubes

Sample: Isapgol (Plantagoovata) seed

Water (q.s)

PROCEDURE

Determination of swelling index

Transfer 1gm of isapgol seed to a 25 ml stoppered measuring cylinder. Fill the cylinder up to 20 ml mark with water. Agitate gently occasionally during 24 hour and allowed to stand. Measure the volume occupied by the swollen. The genuine seed of isapgol occupies a volume of not less than 10 ml.

Determination of foaming index

The foaming ability of an aqueous decoction of plant materials & their extracts are measured in terms of a foaming index.

Test

Weigh accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintain at moderate boiling at 80- 90 C for about 30 min. Then make it cold, filter into a volumetric flask and addsufficient water through the filter to make the volume up to 100 ml (V1).

Cleaned stopper test tubes 10 numbers are taken and marked with 1 to 10. Take the successive portions of 1, 2 ml up to 10 ml drug in separate tubes and adjust remaining volume with the liquid up to 10 ml in each test tube. After closing the tubes withstoppers, Shake them for 15 seconds and allowed to stand for 15 min. then measure the height.

If the height of the foam in each tube is less than 1cm, the foaming index is less than 100 (not significant). Here, if the foam ismore than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube is theindex sought. If the height of the foam in every tube is more than 1 cm, the foaming index is more than 1000. In this case, 10 ml of the first decoction of the plant material needs to be measured and transferred to a volumetric flask of 100 ml capacity (V2) and volumeis to be maintained up to 100 ml and follow the same procedure.

Foaming index is calculated by using the following formula

Foaming index = 1000/a in case of V1

Foaming index = 1000 × 10/a in case of V2

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam is observed.

CONCLUSION

Swelling factor of supplied isapgol seeds is _____ and foaming index is _____.

REFERENCES

1. Kokate CK. Practical Pharmacognosy, 4 edition, VallabhPrakashan. Delhi; 1994: 127.

2. Quality control methods for medicinal plants material by W.H.O. Guidelines : 34.