Experiment No. 7

Aim

Determination of leaf constant such as stomatal index, stomatal number, vein-islet number, vein termination number and palisade ratio.

Reference

1. https://www.pharmatutor.org/articles/evaluation-crude-drugs-mono-polyherbal-formulation?page=2

2. Kokate C.K, Purohit A.P and Gokhale S.B. Nirali Publication, 56th edition

Theory

Stomatal No.

It is average no. of stomata per sq. mm of the epidermis of the leaf, it is affected by various factor like age of plant, size of leaf, environmental condition etc.

Determination of Stomatal Number

Place leaf fragments of about 5x5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40 x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each stomata and calculate the average number of stomata per square millimeter for each surface of the leaf.

Table: Determination of Stomatal Number

Species	number of stomata per sq.mm	
	Upper surface	Lower surface
Atropa belldona	7.5 to 10 to 17.5	77.5 to 113 to176
Cassia angustifolia	180 to 200 to 223	195 to 220 to 257

Stomatal index:

Determination of stomatal index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell. Place leaf fragments of about 5 × 5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover-glass to prevents the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x)for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows:

$$I = \frac{S}{E + S} x\ 100$$

Where

S = the number of stomata in a given area of leaf; and

E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each sample of leaf make no fewer than ten determinations and calculate the average index.

Examples:

Table: Stomatal Index

Species	Stomatal index	
	upper surface	lower surface
atropa belldona	2.3 to 3.9 to 10.5	20.2 to 21.7 to 23.0
cassia angustifolia	17.1 to 19.0 to 20.7	17.0 to 18.3 to 19.3

Vein islet number:

Determination of Vein-Islet Number

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed "Vein- Islets". The number of vein-islets per square millimeter is termed the "Vein-Islet number". This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species.

The determination is carried out as follows:

For Whole or Cut leaves

Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing chloral hydrate solution on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in glycerol-solution or, if desired, stain with safranin solution and prepare the mount in Canada Balsam. Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the center of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make no fewer than three determinations and calculate the average number of vein-islets per square millimeter.

Palisade ratio:

Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells under one epidermal cell. Place leaf fragments of about 5 × 5 mm in size in a test-tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outlines of the four epidermal cells. Count the palisade cells under the four epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the "Palisade ratio" For each sample of leaf make no fewer than ten determinations and calculate the average number.

Palisade ratio =18.4/4=4.5
Examples

Species	Palisade ratio
Atropa belladonna	6 to 10
Cassia angustifolia	5.1 to 7.5
Datura stramonium	4 to 7
Datura tatula	4 to 7

Determination of Vein-let Termination Number

Vein-let termination number is defined as the number of vein-let termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of vein-let.Cleared the piece of the leaf by boiling with chloral hydrate solution forabout thirty minutes.Arranged the camera lucida and drawing board for making drawings to scale.Put stage micrometer on the microscope and using 16 mm objective, drew a line equivalent to 1 mm as seen through the microscope.Constructed a square on Pharmacognostical Evaluation of CedrelaToonaRoxb. Leaves and Fruits. 40 this line. Moved the paper so that the square was seen in the eye piece, in the centre of the field.Put the slide with the cleared leaf (epidermis on the stage).Traced off the veins, which were included within the square, completing the outlines of those islets, which overlapped two adjacent sides of the square.Counted the number of vein-let terminations present within the square. Found the average number of vein-let termination number from the four adjoining squares to get the value for one sq.mm

Result:

The determination of leaf constants such as, stomatal index, stomatal number, vein-islet number, vein termination number and palisade ratio are successfully studied.