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PHARMACOGNOSTICAL STUDY OF KHAYA SENEGALENSIS A. JUSS GROWING IN EGYPT

PART I: Botanical and chemical study of the leaf.

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ABSTRACT

A detailed macro and micromorphological study of the leaves of Khaya senegalensis A. Juss is given to facilitate their identification either in the entire or in the powdered form. Moreover, 5 compounds have been isolated and proved to be α-amyrin, β-amyrin, B-sitosterol, quercetin and rutin. The fatty acids were converted to their methyl esters and analyzed by G.L.C. In addition, a chromatospectrophotometric method was adopted for quantitative estimation of these flavonoids.

INTRODUCTION

Khaya senegalensis A. Juss (Fam: Meliaceae) is a tree commonly growing in tropical west Africa, Sudan and Uganda, newly introduced into upper Egypt[1]. Khaya senegalensis A. Juss is an important tree in West Africa where it yields a good African Mahogany wood[1,2]. The plant is reported to be used widely in Folk medicine as fibrifuge[2], antimalarial[3,4], and also in the treatment of stomach disorders and

venereal diseases[2,5].

Chemically, some authors reported the presence of tannin[2], alkaloids[6], nimbosterol, nimbosterin[7] and gum[8].

Reviewing the current literature revealed that both botanical and chemical study of the leaves were incomplete and fragmentary[1,9]. Therefore, it was deemed of interest to carry out a comprehensive botanical and chemical study on the leaves of the Egyptian plant.

EXPERIMENTAL

Plant Material:

Fresh leaves of K. senegalensis A. Juss growing in different places at Assiut were collected in April 1981. The identity of the plant was kindly confirmed by Dr. A. Fayed, Dept. of Botany, Faculty of Science, University of Assiut.

1- Botanical Study:

a- Macromorphology:

Khaya senegalensis A. Juss is an erect perennial tree attains up to 25 m. in height. The plant have deciduous leaves, usually present at the ends of branches. The plant blooms in summer, in axillary panicles. The flower is whitish in colour with red disc around the ovary. The fruit is globose, woody, 4-valved, persistant capsule. The seeds are flat, oblong-

eleptic and winged

The leaf:

The leaf (Fig. 1) is compound, paripinnate, hernate, petiolate, 15-35 cm. long and formed of 6-12 leaflets. The lamina of the leaflet is oblong-elliptic, 5-10 cm. long, 2.5-4 cm. wide . It is pale green in colour with glabrous surfaces, entire margin, abrubtly pointed apex, papery texture and asymmetric base.

b- Micromorphology:

The Lamina:

A transverse section in the lamina through the midrib (Fig. 2 A) appears oval to slightly biconvex in outline in the midrib region. It shows an upper and lower epidermises enclosing inbetween a dorsiventral mesophyll which is replaced in the midrib region by the vascular strand and the cortical tissue.

The upper and lower epidermises (Fig. 2 D,E,F,G) consist of polygonal cells with nearly straight or curved to slightly wavy anticlinal walls and have thick, smooth cuticle. The upper epidermal cells measure 25-70 μ in length, 20-35 μ in width and 20-38 μ in height. The lower epidermal cells measure, 7-45 μ in length, 8-20 μ in width and 10-22 μ in height. The upper and lower neural epidermal cells are axially elongated with nearly straight anticlinal walls. The upper neural epidermis measure 20-45 μ in length, 10-30 μ in width and 15-25 μ in height and the lower neural epidermal cells measure 25-65 μ in length, 10-55 μ in width and 10-25 μ in

height.

Stomata of anomocytic type are distributed on the lower surface, being oval, occasionally rounded in shape, surrounded by 4-6 cells and measure 20-35 μ in diameter. The stomatal index vary from 7-9.

Small cluster crystals of calcium oxalate, measuring 8-18 μ in diameter are present in some cells of both upper and lower epidermises. Covering tricomes are absent.

The mesophyll (Fig. , A,C) consists of 2 rows of palisade cells next to the upper epidermis only. The cells are columner in shape and those of the upper row are longer than those of the inner one. The palisade ratio is 3-3.7. The spongy tissue is formed of thin-walled, rounded to ovoid parenchyma,occasionally containing cluster crystals of calcium oxalate measuring 10-25 μ in diameter.

The cortical tissue (Fig. 2 A.B) consists of an upper and lower subepidermal masses of collenchyma, the upper being formed of 4-6 rows and the lower of 2-4 rows of cells . The rest of the cortical tissue is formed of rounded or slightly irregular parenchymatous cells. Some cells of the cortical tissue contain cluster crystals of calcium oxalate measuring 15-30 μ in diameter.

The vascular tissue (Fig. 2 A,B) in the midrib is represented by two separate collateral vascular bundles, the upper of which is inverted. The pericycle is formed of upper and lower arcs of numerous groups of fibres separated by thin walled parenchyma.

The fibres have moderately thick, lignified walls, narrow or wide lumena and blunt or forked tips measuring 520-850 μ in length and 10-20μ in width.

The phloem consists of thin walled soft cellulosic elements containing occasional small clusters of calcium oxalate measuring 5-12 μ in diameter.

The xylem is radiating, composed of vessels, fibres and wood parenchyma and traversed by uni-or biseriate medullary rays. The vessels are lignified with spiral, annular rarely pitted and reticulate thickening, measuring 7-50 μ in diameter. The wood fibres have thin or moderately thick lignified walls, narrow or wide lumena and blunt or tapering ends, measuring 610-900 μ in length and 10-38 μ in width.

The Petiolule:

A transverse section of the petiolule (Fig. 3 E) is more or less circular in outline. Its structure resembles that of the lamina in the midrib region but shows a pericycle formed of a ring of numerous groups of fibres separated by parenchyma and a complete ring of vascular tissue surrounding a central pith.

The Rachis

A transverse section in the rachis is nearly circular in outline (Fig. 3 A) showing an outer epidermis surrounding the cortex, followed internally by a pericycle formed of fibres interrupted by parenchyma and enclosing a complete ring of vascular tissue with narrow pith in the centre.

The epidermis (Fig. 3,C) consists of subrectangular, axially elongated cells with nearly straight anticlinal

walls and thick smooth cuticle. They measure 18-33 μ in width and 15-30 μ in height. Stomata and covering trichomes are not observed.

The cortical tissue is formed of a continuous zone of 3 to 5 rows of collenchymatous cells, followed by a parenchymatous layer with moderately wide intercellular spaces. Numerous cluster crystals of calcium oxalate are present in the cells of the cortical tissue, measuring 10-45 μ in diameter.

The pericycle is formed of numerous large groups of fibres forming a ring interrupted by parenchyma. The pericyclic fibres are similar to those of the midrib of the leaves, they measure 530-840 μ in width.

The phloem consists of thin walled soft shining cellulosic elements traversed by bi or triseriate medullary rays with slightly lignified and pitted walls. The vessels are lignified with spiral, annular rarely pitted or reticulate thickening, measuring 23-80μ in diameter. The wood fibres are straignt or slightly irregular in outline. They have wide lumena, tnick lignified walls, showing oblique slit-likepits and tapering or blunt apices. They measure 560-1000 μ in length and 25-48 μ in width. The wood parenchyma are nearly rectangular, axially elongated having pitted lignified walls.

The pith consists of large, thin-walled parenchymatous cells with moderately wide intercellular spaces. Numerous cluster crystals of calcium oxalate are present in the pith.

Powder :

The powdered leaf is pale green in colour with faint odour and a bitter taste. It shows:

1- Fragments of the epidermis consisting of polygonal cells with nearly straight or curved to slightly wavy anticlinal walls, a smooth cuticle and showing anomocytic stomata and small cluster crystals of calcium oxalate.

2- Fragments of the epidermis of the neural region or of the rachis, formed of polygonal, axially elongated cells.

3- Fragments of spiral, annular rarely pitted and reticulate vessels.

4- Fragments of collenchymatous and parenchymatous cells of the cortical tissue, occasionally containing cluster crystals of calcium oxalate.

5- Fragments of the lamina showing palisade cells.

6- Fragments of wood parenchyma of the rachis formed of axially elongated cells with pitted lignified walls.

7- Numerous fragments of fibres with lignified walls narrow or wide lumena and acute, blunt or forked apices.

8- Fragments of the wood fibres with thick lignified walls, wide lumena, tapering or blunt apices showing oblique slit-likepits.

9- Numerous free cluster crystals of calcium oxalate.

10- Sclereids and trichomes are absent.

II- Chemical Study:

Preliminary Phytochemical Examination:

The preliminary phytochemical studies of the leaves of K. senegalensis A. Juss. proved the presence of sterols and/or triterpenes, flavonoids, tannins and carbohydrates and/or glycosides.

Extraction and Fractionation:

The air-dried powdered leaves of K.senegalensis A. Juss (5 Kg.) were successively extracted with pet.ether(b.r.60-80 °C), chloroform and finally ethanol 70%. Each extract was separately concentrated under reduced pressure and fractionated as follows:

Study of Lipids:

The pet. ether extract (20 g.) were subjected to the usual method of saponification using 0.5 N alcoholic KOH[10]

The unsaponifiable matter after evaporation of ether was screened on silica gel (E. Merck) plate using chloroform-methanol (99.5 : 0.5) as a solvent system and methanolic sulfuric acid for location of spots (after heating at 110° for 5 min.).

Only 7 spots were located, three of them have R_f values similar to authentic of α-amyrin (R_f 0.48), B-amyrin(R_f0.44) and B-sitosterol (R_f 0.36).

The unsaponifiable matter was fractionated on a column of neutral alumina and eluted with pet. ether and then pet.ether containing increasing amounts of ethyl acetate viz. 5,10 and 40% to yield α-amyrin (123 mg, m.p. 183-186°C), B-amyrin (58 mg, m.p. 197-198°C) and B-sitosterol (180 mg, 135-138°C).

The I.R., m.p. and mixed m.p. of the three compounds were found to be identical with those reported for authentic α-amyrin, B-amyrin and B-sitosterol.

Fatty Acids:

The mother liquor after extraction of unsap. was rendered acidic with dil. hydrochloric acid and extracted with ether (6 x 200 ml) to give the free fatty acids. **Methylation of the fatty acids** was done using methanol and sulfuric acid[11]. The methyl esters were extracted with ether, concentrated and analysed by GLC (Pye unicam GLC Model 64, Series 104, England, equiped with a dual flame ionisation detector), adopting the following operating conditions:

Column/coiled glass, 2 m long, 5 mm. i.d., packed with 10% polyethylene glycol adipate on chromosorb P (60-80 mesh), column temp. 190°C, injection port temp 220°C, detector temp. 250°C, the flow rates of Hydrogen, air and nitrogen were 50, 400 and 45 ml per minute respectively, chart speed 5 mm/min.

Qualitative identification was based on the relative time of the resolved peaks compared with the authentic samples analyzed under the same conditions.

Quantitative analysis was based on the internal normalization method using the peak area measured by triangulation.

The results are listed in Table 1

Study of Flavonoids:

A- Chloroformic Extract:

TLC of the chloroformic extract of the air dried leaves on silica gel G plates using chloroform-methanol-formamide (80:19:1) as a solvent system I and aluminium chloride in ethanol as spraying reagent for location of the spots (after heating at 100°for 5 min.) revealed the presence of only one spot R_f(0.80).

Isolation and purification of this flavonoid was achieved by small column chromatography (silica gel for column, Merck) eluted with chloroform-methanol mixture. The eluate chloroform 10% was concentrated under reduced pressure and the residue was dissolved in methanol 70% and left for crystallisation, where yellowish crystals were obtained m.p. 316-318°C, U.V. (MeOH) 255,370 nm; + NaOAC 272,382 nm; + $NaOAC/H_3BO_3$ 262,293 nm, + $AlCl_3$ 269, 337, 458 nm.

These properties were found identical with those reported for quercetin[12]. The identity was further confirmed by means of co-chromatography and mixed melting point with authentic quercetin .

B- Alcoholic Extract:

TLC of the alcoholic extract on silica gel G plates using solvent system I and also paper chromatography on Whatman No. III using n-butanol-acetic acid-water(4:1:2) as solvent system (system 2) revealed the presence of only one spot having R_f(0.16 & 0.55) respectively.

Isolation of the flavonoid was achieved by concentration of the alcoholic extract under reduced pressure and the residue dissolved in acetone-water mixture (3:1) and left over-night, where amorphous yellowish substance was left out. The amorphous substance was filtered off and

crystallised from methanol to yield yellowish white crystals, m.p. 193-195°C UV λ_{max} (MeOH) 258, 265 sh, 360 nm. + NaOAC 272, 381 nm; + NaOAC/H_3BO_3 266, 384 nm.; + NaOAC 275, 421 nm; + Al Cl_3 275, 433 nm.

This compound was identified as rutin by direct comparison with authentic rutin sample and by the detection of quercetin, rhamnose and glucose after hydrolysis.

Quantitative Study:

A- Estimation of Total Flavonoids Calculated as Rutin:

Twenty grammes of the air-dried powdered leaves (P)were successively extracted with pet.ether(b.r.40-60°), chloroform & methanol in soxblet apparatus. The methanolic ext. was concentrated to 25 ml(V), 0.2 ml of the ext.(V_1) was chrmoatographed on PC(3 MM) using system n-butanol:acetic acid:water(4:1:2). Flavonoidal spots were cut, eluted with methanol and the eluate was concentrated to 25 ml (W). The absorbanc of the eluate was determined at 359 nm. and the corresponding concentration(C, mg%) was calculated from the standard curve of rutin. Percentage(g% w/w)of total flavonoids(X) calculated as rutin was deduced from the equation:

$$X = \frac{W.C.V.\ \ 100}{P.V_1.E.\ 1000}$$

where E=percentage of elution of rutin from PC and was found to be 80.0%.

B- Estimation of Rutin:

Following the same procedure for total flavonoids, elution was done only for the spot corresponding to rutin (spot with R_f 0.50). The absorbance of the eluate was determined at 359 nm.

Percentage of total flavonoids was found to be 0.55 g% w/w.

Percentage of rutin was found to be 0.45 g% w/w.

It is clear that the flavonol rutin is the major constituent since it constitutes about 82% of the total percent of flavonoids of the leaves.

The assay was done in triplicate.

Table 1: Fatty acids, their relative retention times and approximate percentage compoition.

Peak No.	*Fatty acids*	*r**	*% of Fatty acids*
1	Lauric	0.13	25.20
2	Myristic	0.38	10.08
3	Palmitic	1.00	33.61
4	Stearic	1.20	traces
5	Oleic	1.5	3.36
6	Linoleic	1.9	4.20
7	Linolenic	2.3	23.52

r* = Retention time relative to that of palmitic acid

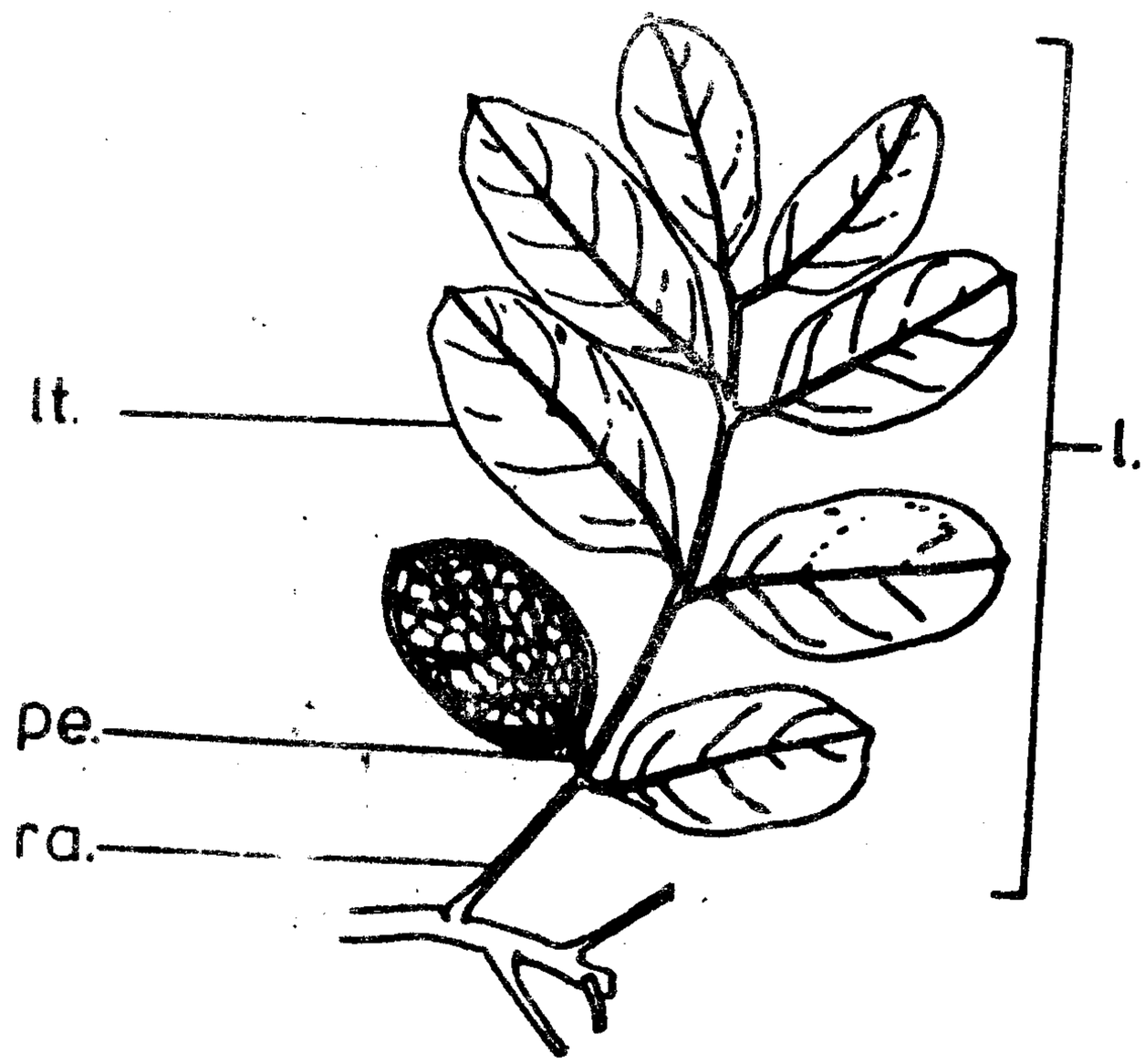

Fig. 1: The leaf. X ½

l., leaf; lt., leaflet; e., petiolule; ra., rachis.

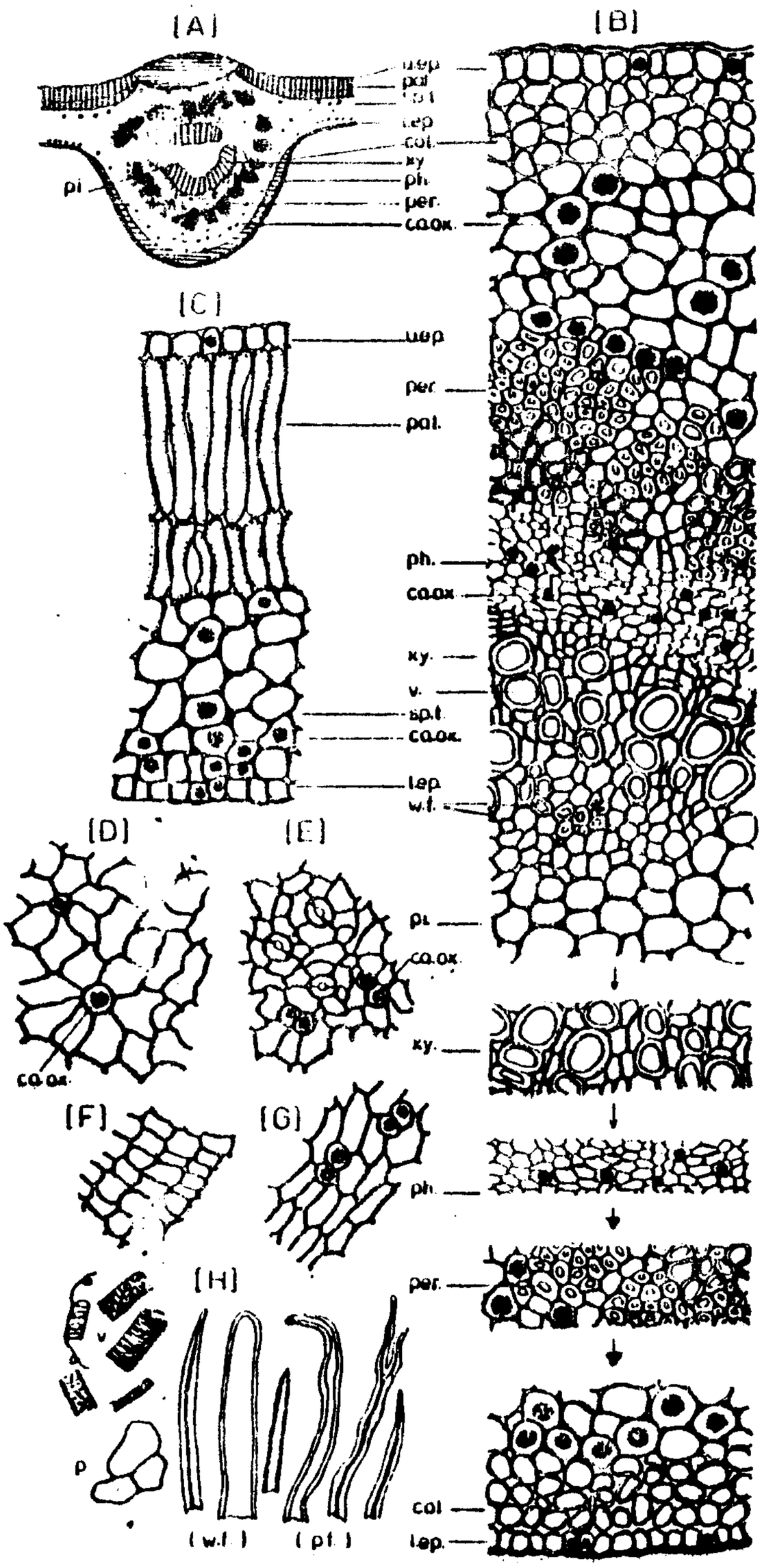

Fig. 2: The Leaf

A.	**Diagrammatic T.S. of the leaflet**	**X 17**
B.	**Detailed T.S. of the midrib**	**X 210**
C.	**Detailed T.S. of the lamina**	**X 210**
D.	**Upper epidermis of the lamina**	**X 210**
E.	**Lower epidermis of the lamina**	**X 210**
F.	**Upper neural epidermis**	**X 210**
G.	**Lower neural epidermis**	**X 210**
H.	**Isolated elements of the leaf**	**X 210**

ca.ox., calcium oxalate crystals; col., collenchyma; l.ep., lower epidermis; pal., palisade tissue; p., parenchyma; per., pericycle; p.f., pericyclic fibre; ph., phloem; pi., pith; sp.t., spongy tissue; u.ep., upper epidermis; v., vessels; w.f., wood fibre; xy., xylem.

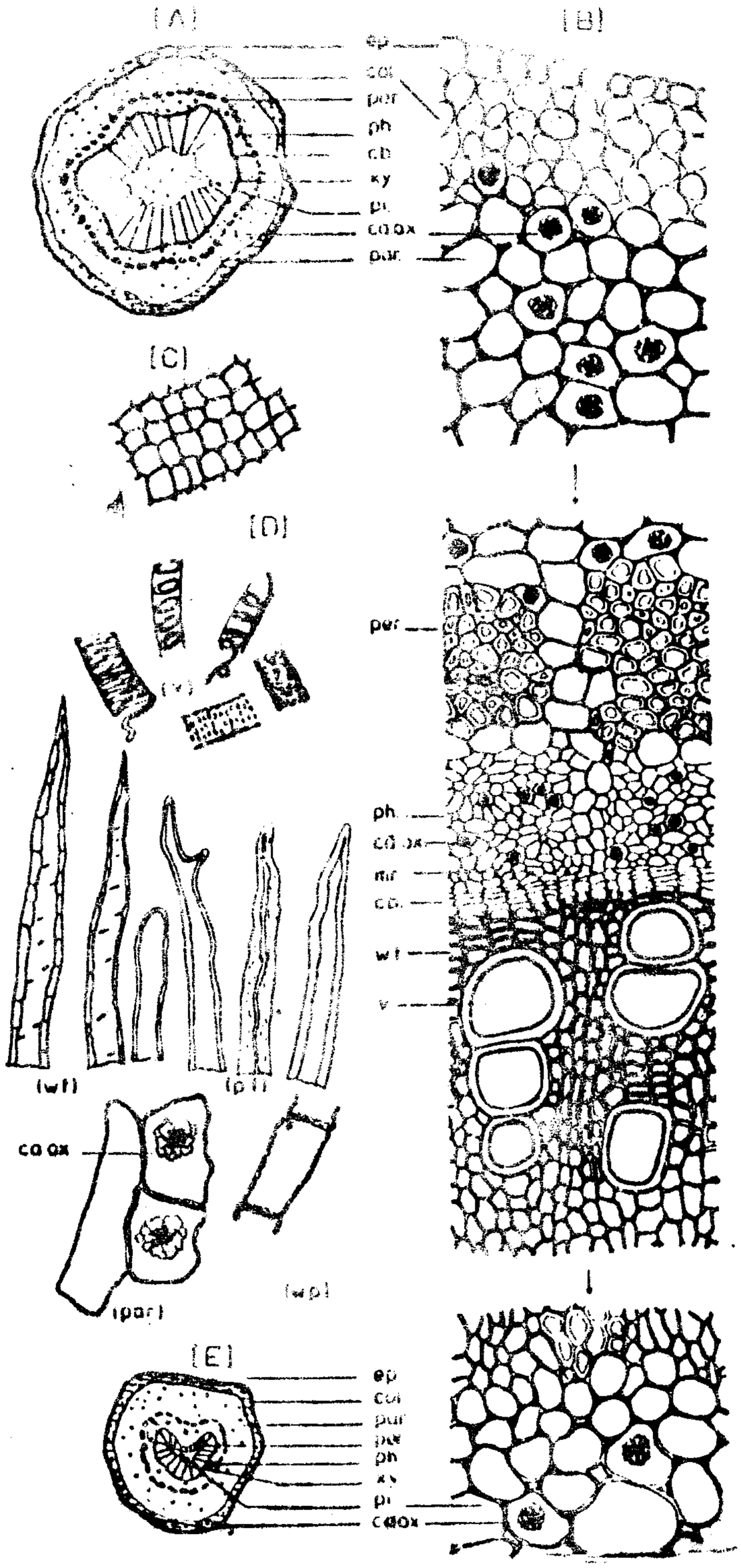

Fig. 3: A. Diagrammatic T.S. of the rachis X 13
B. Detailed T.S. of the rachis X 210
C. Surface preparation of the rachis X 210
D. Isolated elements of the rachis X 210
E. Diagrammatic T.S. of the petiolule X 13

ca.ox., calcium oxalate; cb., cambium; col., collenchyma; ep., epidermis; m.r., medullary ray; par., parenchyma; per., pericycle; p.f., pericyclic fibre; ph., phloem; pi., pith; v., vessel; w.f., wood fibre; w.p., wood parenchyma; xy., xylem.

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دراسة عقاقيرية لاوراق نبات الكاياسينجاليينس المنزرع في مصر

مصطفى كامل مصباح - محمد عبد المطلب عبد الحافظ
محمد احمد عبد الرحمن الشنوانى
قسم العقاقير - كلية الصيدلة - جامعة اسيوط

١ - فى هذا البحث اجريت دراسة عيانية ومجهرية لاوراق نبات الكاياسينجاليينس وهذه الدراسة تساعد على التعرف عليها سواء كانت كاملة او على هيئة مسحوق .

٢ - تمت دراسة المحتوى الدهنى باستعمال كروماتوجرافيا الطبقة الرقيقة وكروماتوجرافيا العمود وكروماتوجرافيا الغاز عما ياتى :

ا - وجود البيتاساسيتوستيرول - الفا اميرين فى الجزء الغير قابل للتصبن .

ب - احماض دهنية مشبعة وهى لوريك - ميريستيك - بالمتيك - استياريك - واحماض دهنية غير مشبعة وهى اوليك - لينوليك فى الجزء القابل للتصبن .

٣ - تمت دراسة المواد الفلافونيدية وإمكن فصل والتعرف على مادتى الروتين والكوارسيتين .

٤ - تبين ان النسبة المئوية الكلية لمركبات الفلافونيدية هى ٠/٠و٥٥ بينما النسبة المئوية لمادة الروتين تصل الى ٠/٠و٤٥ .

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