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PHARMACOGNOSTICAL STUDY OF PORTULACA OLERACEA L. GROWING IN EGYPT

Part I: Botanical study of the Stems, Leaves and investigation of the lipid content.

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ABSTRACT

The macro and micromorphology of the stem and leaves of Portulaca oleracea L. growing in Egypt are presented with the aim of finding their characters by which they could be identified and differentiated. Moreover, the herb was subjected to phytochemical screening and the lipid content was investigated.

INTRODUCTION

Portulaca oleracea L. (Fam. Portulacaceae or Pirslane Family)[1,2,3] is a common annual weed in the fields, mainly distributed in the tropical and temperate regions of the world[2,3]. It is often used as a potherb and salad,[2,4] as cooling diuretic[5], as sudorific[6], and anti-scorbutic for skin conditions[7]. It has been regarded as being tonic, also used in snak-bite remedy[8],

in pulmonary diseases and in haemoptysis[8]. In south Africa it is used as a charm[9] and is eaten as cooked vegetable[4-11]. The seeds have anthelmintic effect on ascaris[12]. The Australians suspected that the plant have a poisoning effect on cattles and sheep[7].

The plant has attributed only scant chemical interest. Kolev et al stated the presence of traces of alkaloids, coumarins, flavones, B-sitosterol and slight positive test for hydrocyanic acid[13]. Handa et al (1956)[14] stated that the seeds of the plant contained 17.4%. oil. The oil consists of 18.1% solid and 81.9% liquid acids. The acids are palmitic 10.89, stearic 3.71, behenic 1.28 , oleic 28.69, linoleic 38.9 and linolenic 9.9%. They reported the presence of B-sitosterol in the seeds of the plant.

The present work deals with preparation & identification of the lipid content of the herb of Portulaca oleracea L. as well as the macro-and micromorphology of the stems and leaves.

Habitat:

Portulaca oleracea L. is an annual weed common in field during summer season and sometimes cultivated. The herb(Fig. 1,A) attaining 10-40 cm in height and has ascending herbaceous stems. It bears green, alternate, sessile fleshy leaves and inflorescence of numerous small, yellow sessile flowers in forks. It gives flowers through greated part of the season. The fruits are small capsule containing numerous seeds and dehiscing transversely.

Pharmacognostical study of Portulaca oleracea L. growing in Egypt

Part 1: Botanical study of the stems, leaves and investigation of the lipid content

EXPERIMENTAL

Plant Material:

The plant material was collected in June 1982 from plants growing wild around Assiut University campus.

The identity of the plant was verified by Dr. N.E. El-Keltawi, associate professor of Floriculture, Faculty of Agriculture, Assiut University.

I. BOTANICAL STUDY

MACROMORPHOLOGY

1- The Stem: (Fig. 1,A)

The stem of the mature plant is ascending sometimes prostrate, herbaceous, succulent, cylindrical to subcylindrical in outline, solid, reaching about 10-30 cm in length and up to 0.3-1 cm in diameter near the ground level. It carries more or less short internodes measuring about 2-4 cm in length. The stem is smooth, green in colour with violet tinge and slightly longitudinally striated;it shows monopodial branching. It has a faint odour and characteristic mucilagenous taste.

2- The Leaf: (Fig. 1,A & B)

The plant carries alternate to sub-opposite, exstipulate, simple, sessile leaves clustered at the ends of the branches. The leaf is spathulate to oblong-obovate in shape with entire margin, abtuse to retuse apex, fleshy texture and symmetric base. The leaves

measure about 0.8-3 cm in length and 0.5-1.3 cm in width at the widest part. Both surfaces are glabrous, the upper surface is dark green in colour with a violet margin while the lower one is lighter. Venation is pinnate reticulate. The leaf possesses a faint odour and a mucilagenous taste.

MICROMORPHOLOGY

1- The Stem:

A transverse section through the stem (Fig. 2,A) is nearly circular in outline. It shows a glabrous outer epidermis enclosing a comparatively narrow collenchymatous hypodermis followed by parenchymatous cortex. The endodermis is distinct and starchy. The pericycle consists mainly of parenchyma cells, surrounds the central cylinder which is formed of bundles of vascular elements. Each vascular bundle consists of an external phloem and internal radiating xylem. The phloem and xylem are traversed by narrow uni-or biseriate medullary rays. The pith is centric and solid.

The Epidermis:(Fig. 2,B & Fig. 3) appears in transverse section as one row of square to subrectangular cells. In surface view (Fig. 2,B) the cells appear polygonal nearly square somewhat axially elongated with straight anticlinal walls and measure from 130 to 300 µ in length, 100 to 180 µ in width and 30 to 50 µ in height.

Pharmacognostical study of Portulaca oleracea L. growing in Egypt
Part 1: Botanical study of the stems, leaves and investigation of the lipid content

The epidermal cells are covered with somewhat thick smooth cuticle. No stomata or trichomes are observed.

The Cortex:(Fig. 2,A & 3) shows an outer layer of 2 to 3 rows of more or less rounded to oval collenchymatous cells. These are followed by 5-9 rows nearly rounded parenchyma cells; sometimes containing chloroplasts and mucilage that stains red with Ruthenium red (T.S.). They also contain cluster and prisms of calcium oxalate measuring 50 to 80 μ in diameter and 18 to 45 μ in length respectively.

The endodermis consists of conspicuous layer of tabular thin-walled cells, containing starch granules either simple or compound of 2-5 components.

The Pericycle: (Fig. 3) consists of round or oval thin-walled parenchymatous cells with narrow intercellular spaces.

The Vascular System: (Fig. 2,A & Fig. 3) the individual vascular bundle shows an outer phloem formed of sieve tubes, companion cells and phloem parenchyma, followed by narrow indistinct cambium. The xylem consists of lignified reticulate, annular and spiral vessels accompanied by non-lignified wood parenchyma. They measure from 30 to 60 μ in diameter. The medullary rays are mostly biseriate, sometimes uniseriate. These cells are subrectangular with non-lignified walls in the phloem and xylem region.

The pith is formed of somewhat large, rounded, isodiameteric, thin-walled parenchyma, The cells contain mucilage, prisms and cluster crystals of calcium oxalate which are larger than those of the cortex. The prisms measure from 20 to 55 μ in length and the clusters from 65 to 110 μ in diameter.

The Powder: (Fig. 2C)

Powdered stem of Portulaca oleracea L. is dark green in colour having a faint odour and mucilagenous taste. It is characterised microscopically by the following:

1- Fragments of polygonal, nearly square epidermal cells with straight anticlinal walls and covered with thick smooth cuticle.

2- Fragments of thin-walled parenchymatous cells from the cortex containing chloroplasts.

3- Fragments of lignified vessels with reticulate, annular and spiral thickening.

4- Fragments of thin-walled parenchyma cells of the pith containing prisms and cluster crystals of calcium oxalates.

5- Absence of sclereids and hairs.

2- The Leaf:

A transvers section in the lamina through the midrib (Fig. 4,C) is somewhat planoconvex in outline. It shows an upper and lower epidermises enclosing a dorsiventral heterogenous mesophyll traversed by several collateral vascular bundles. Each bundle is surrounded by a layer of colourless parenchyma cells and another one

of green palisade cells[4].

The Epidermis: (Fig. 4,A,B &D) the upper and lower epidermal cells of the lamina are polygonal nearly isodiametric to slightly elongated with wavy anticlinal walls and covered with thin smooth cuticle. They contain mucilage which stains red with Ruthenium red(T. S.). The cells of both epidermises are nearly equal in size and measure from 150 to 300 µ in length, 80 to 200 µ in width and 50 to 95 µ height. Stomata of rubiaceous type are present on both surfaces (accompanied by 4 subsidiary cells parallel to the pore)[4]. They are most numerous on the lower surface, measuring 45 to 80µ in length and 30 to 60 µ in width. No hairs are observed.

The mesophyll:(Fig. 4,D) the palisade forms an upper zone of one row of comparatively large columnar cells some of them containing chloroplasts, measuring from 60 to 190 µ in length and 48 to 90 µ in width . The spongy mesophyll is formed of thin-walled irregular parenchyma with intercellular spaces, containing cluster and prisms of calcium oxalate, they measure 35 to 70 µ in diameter and 18 to 40 µ in length respectively.

The cortical tissue: (Fig. 5,A) consists of upper and lower parenchyma cells with narrow intercellular spaces sometimes containing chloroplasts and calcium oxalate crystals.

The vascular system: (Fig. 5,A)consists of several radially arranged collateral vascular bundles surrounded by

narrow parenchymatous pericycle above and below the vascular bundles. The phloem is formed of soft cellulosic elements showing sieve tubes, companion cells and phloem parenchyma. The xylem consists of reticulate and spiral lignified vessels measuring from 18 to 50μ in diameter. Medullary rays are uni-sometimes biseriate and non lignified.

The Powder: (Fig. 5B)

The powdered leaves are dark green in colour with a faint odour and a mucilagenous taste. Microscopically, it is characterised by the following:

1- Fragments of epidermal cells which are polygonal, nearly isodiametric with wavy anticlinal walls and covered with thin smooth cuticle. These fragments carry rubiaceous stomata.
2- Fragments showing large and small palisade cells, in addition to spongy parenchyma containing cluster and prisms of calcium oxalates.
3- Lignified spiral and reticulate xylem vessels.
4- Absence of hairs and sclereids.

II. PHYTOCHEMICAL STUDY

Preliminary Phytochemical Screening:

The plant under investigation was subjected to phytochemical screening[15]. This study revealed the presence of carbohydrates and/or glycosides, alkaloids and/or basic nitrogenous components, sterols and/or triter-

penes and flavonoids.

Extraction and Fractionation:

Five kg. of the fresh herb of P.oleracea L. were extracted by percolation with EtOH 90%. The extract was concentrated and defatted with pet.ether (b.r. 60-80°C). The pet.ether extract was concentrated under reduced pressure and subjected to fractionation.

1. Study of the Lipids:

The pet.ether extract (50 g.) was saponified using 0.5 N alcoholic KOH[16].

The unsaponified matter was extracted with several successive portions of ether till exhaustion. The remaining alkaline aqueous solution was acidified with dil. H_2SO_4. The liberated fatty acids were extracted with ether (5 x 50 ml.). The combined ethereal extracts were washed with distilled water, dehydrated over anhydrous Na_2SO_4 and the ether was distilled off.

Fatty Acids: The obtained fatty acids (11 g.) were converted to their methyl esters[17] and analysed by G L C (Perkin Elmer Sigma 3-B. gas chromatograph, equiped with FID) adopting the following conditions:

Column	:	coiled glass, 2m. Long, 5mm. i.d.,
Packing material	:	3% OV-17.
Oven temp	:	190°
Injection port temp.	:	250°
Detector temp.	:	250°
Carrier gas	:	nitrogen.

Flow rate of hydrogen : 50
Flow rate of air : 400
Flow rate of nitrogen : 50
Chart speed : I cm/min.
Attenuation : 16 x 10
Sample size : I µL.

Identification of the fatty acids under investigation was accomplished comparing in each case the relative retention times of their methyl esters with those of pure available authentics. Quantitative analysis was carried out based on peak area measurements.

The results obtained are listed in the following table

The Unsaponifiable Matter: It was examined by TLC on silica gel G(E.Merck) plates using chloroform as a solvent system and methanolic sulfuric acid for location of spots (after heating at 110°C for 5 min.). Only 5 spots were located(with R_f values : 0.93, 0.49, 0.41,0.39 and 0.25); two of them (R_f0.41 & 0.25) are chromatographically identical with-B-amyrin and B-sitosterol respectively.

The unsaponifiable matter (5 g.) was chromatographed over alumina column (1 kg. 120 x 5 cm) using solvent petroleum-ether, pet. ether-chloroform, chloroform and finally chloroform-ethanol. Fractions (1 liter each)were collected, separately concentrated and examined in TLC using $CHCl_3$ as solvent system. Three compounds were

isolated in sufficient amounts and designated 1-3.

Compound 1:

White flakes (23 mg) from acetone, m.p. 55-57°C. (R_f=0.93, silica gel G, solvent: chloroform). It did not decolourise bromine water suggesting saturation and gave negative Salkowiski's and Liebermann Burchard's tests.The IR spectrum revealed no characteristic absorption bands except those due to saturated C-H stretching and bending vibrations and this indicating its paraffinic nature.

Compound 2:

White microcrystals (30 mg) from methanol, m.p. 200-202°C, (R_f=0.41, silica gel G; solvent; chloroform) acetate m.p. 240-242°C m.p., mixed m.p. and superimposable I R spectra with authentic B-amyrin confirm its identity.

Compound 3:

White feathery needles (350 mg) from $CHCl_3$, m.p. 136-138°C, R_f = 0.25, silica gel G, solvent: chloroform, acetate m.p. 125-127°C. Its identity as B-sitosterol was confirmed by m.p., mixed m.p. and superimposable IR spectra. TLC of the acetate derivatives of compound 3 on argentized silica gel G[18] wedge shaped plates using solvent system pet.ether-chloroform-glacial acetic acid (15:25:0.5) proved to be a mixture of 3 spots with R_f 0.23, 0.31 and 0.35 coinciding respectively with campesterol, stigmasterol and B-sitosterol acetates.

RESULTS AND DISCUSSION

Portulaca oleracea is one of the Portulaca species grown in Egypt. A preliminary screening of the plant was undertaken. The GLC analysis of the fatty acids revealed the presence of 11 fatty acids of which 8 fatty acids could be identified. Palmitic, capric and lauric acids were the most prominent(30.11, 19.00 and 16.91% respectively); myristic and linoleic acids were of moderate concentration (10.45 and 5.94% respectively) while oleic and stearic acids were detected as minor compounds (2.08 and 1.43% respectively).

The unsap. matter of the herb was found to contain B-sitosterol, stigmasterol, campesterol and B-amyrin. In addition, the phytochemical screening of the herb revealed the presence of the alkaloids and/or basic nitrogenous substances in trace amount. Chromatographic analysis of the conc. chloroformic extract of these substances revealed the presence of three Dragendorff- positive spots of R_f values 0.75 (A), 0.33 (B) and 0.13 (C) (TLC, silica gel G, $CHCl_3$ CH_3OH, 9:1). Substance (B) which is the major one was separated by preparative TLC. Work is in progress for further purification and identification of these substances. Moreover, a detailed macro and micro-morphology of the stems and leaves were undertaken with view of finding the characters which facilitate their identity in both the entire and powdered forms.

Results of GLC analysis of the methyl esters of the fatty acids separated from lipids of P. oleracea L.

Peak No.	*Fatty Acid Methyl Ester*	*R**	*% of Fatty Acid*
1	Capric	0.15	19.0
2	Lauric	0.30	16.91
3	Myristic	0.36	10.45
4	Isopalmitic	0.66	5.94
5	Palmitic	1.0	30.71
6	Palmitoleic	1.26	3.79
7	Stearic	1.60	1.43
8	Oleic	1.72	2.08
9	Linoleic	2.03	7.84
10	Unknown	2.67	1.48
11	Unknown	3.28	0.36

* Retention time relative to that of palmitic acid.

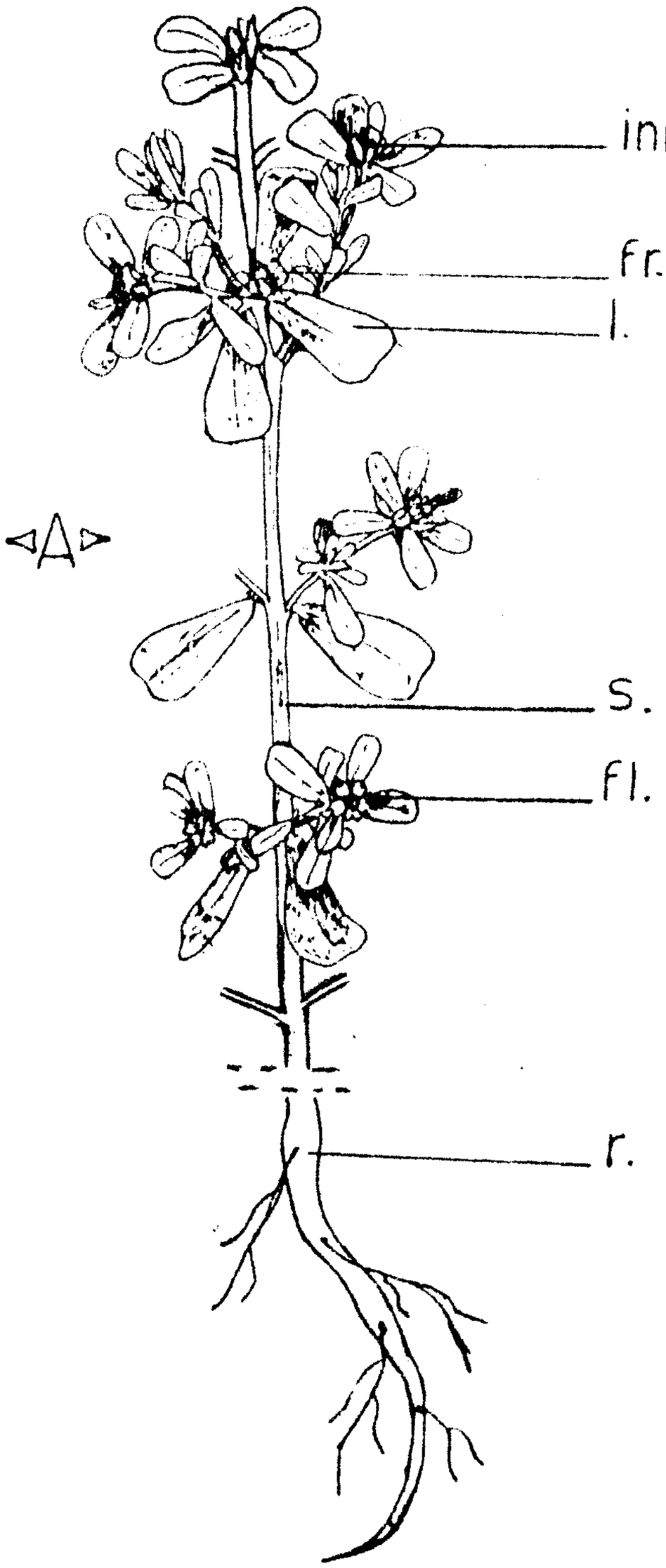

Fig. 1: A- Sketch of Portulaca oleracea L. X $\frac{1}{2}$
B- The leaf
fl., flower; fr., fruit, inf., inflorescence; l., leaf; r., root; s., stem.

Pharmacognostical study of Portulaca oleracea L. growing in Egypt
Part I: botanical study of the stems, leaves and investigation of the lipid content

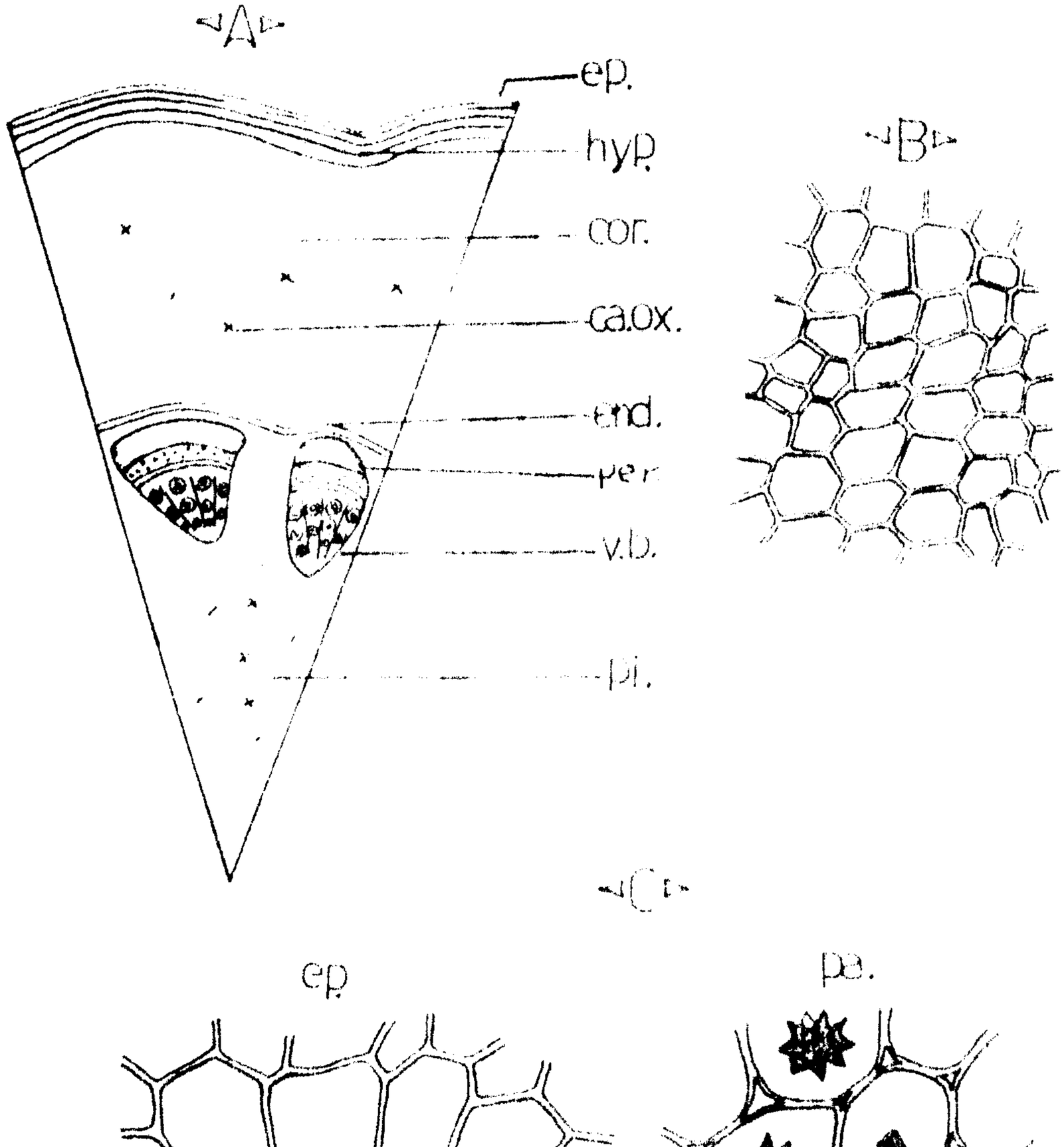

Fig. 2: A- Diagrammatic T.S. of the stem X 50
B- Surface preparation of the stem X 90
C- Isolated elements of the stem X 150
ca.ox., calcium oxalate; coll., collenchyma; cor., cortex; cr., crystal; end., endodermis; ep., epidermis; hyp., hypodermis; pa., parenchyma; pi., pith; v.b., vascular bundle; v., vessel.

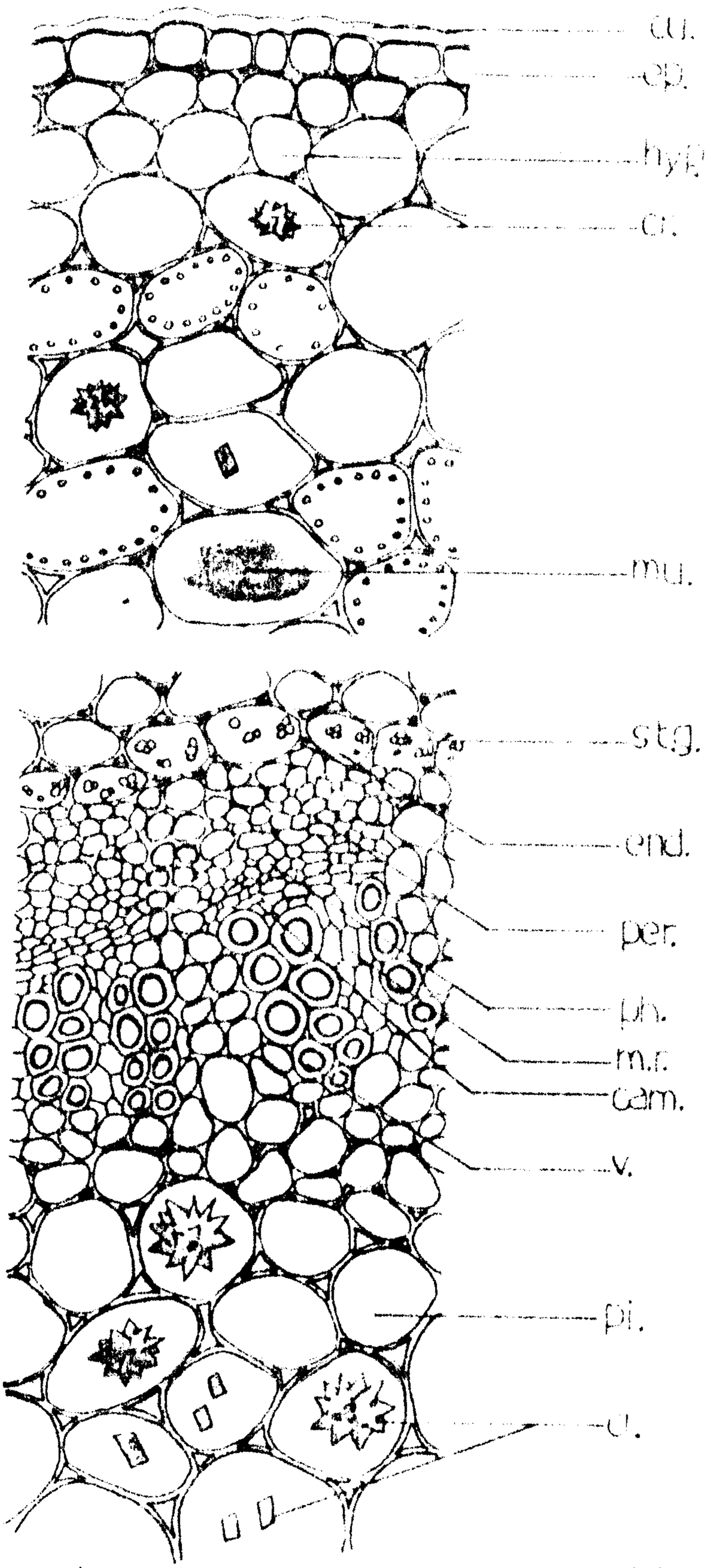

Fig. 3: Detailed T.S. of the stem X 150

cam.,cambium; cr., crystal; cu., cuticle; end., endodermis; ep., epidermis; hyp., hypodermis; m.r., medullary ray; mu., mucilage; per., pericycle; ph., phloem; pi., pith; st.g., starch granule; v., vessel.

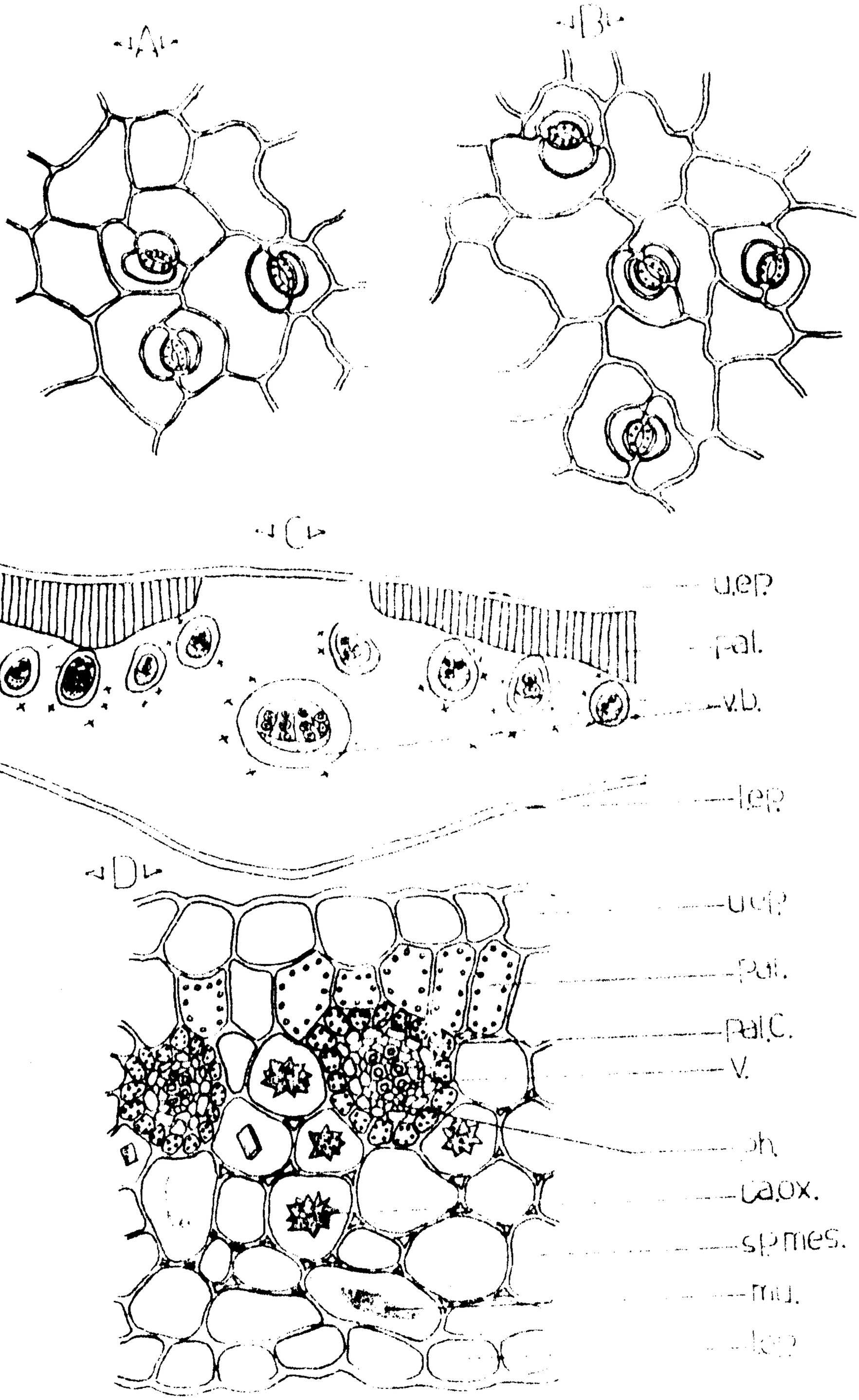

Fig. 4: A- Surface preparation of the leaf (upper epidermis) X 100
B- Surface preparation of the leaf (lower epidermis) X 100
C- Diagrammatic T.S. of the leaf X 60
D- Detailed T.S. of the leaf lamina X 150
ca.ox., calcium oxalate; l.ep., lower epidermis; mu., mucilage; pal., palisade; pal.c., palisade cells; ph., phloem; sp. mes., spongy mesophyll; st., stomata; u.ep., upper epidermis; v.b.,

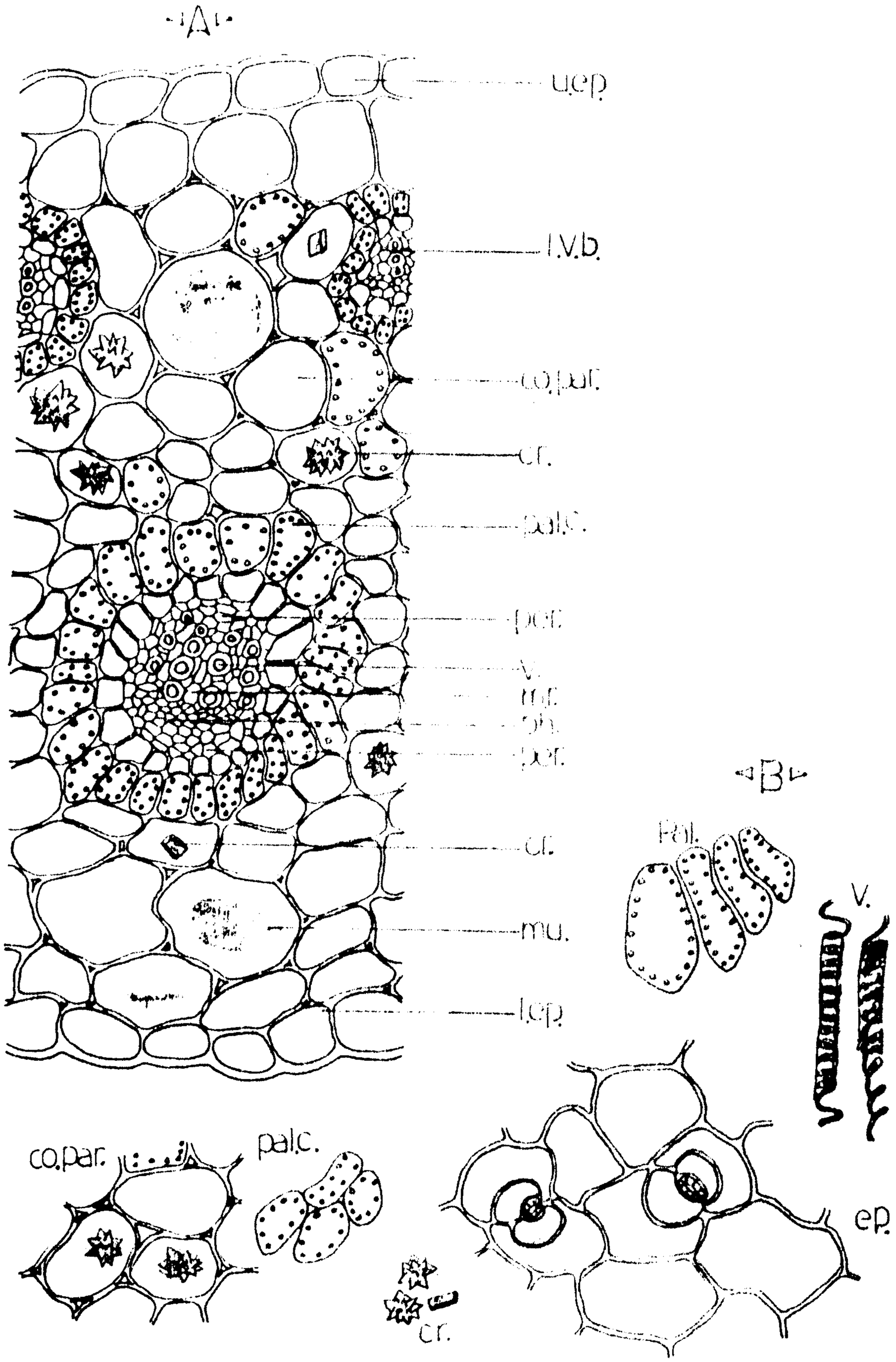

Fig. 5: A- Detailed T.S. of the midrib region X 150
B- Isolated elements of the leaf X 150

co.par.; cortical parenchyma; cr., crystal; ep., epidermis; l.ep., lower epidrmis; l.v.b., lateral vascular bundle; mu., mucilage; m.r., medullary ray; pal., palisade; pal.c., palisade cell; per., pericycle; ph., phloem; u.ep., upper epidermis; v., vessels.

Pharmacognostical study of Portulaca oleracea L. growing in Egypt
Part 1: Botanical study of the stems, leaves and investigation of the lipid content

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دراسة عقاقيرية لنبات البورتلاكا اوليراسيال" الرجلة "
الذى ينمو فى مصـــر

الجزء الاول: الدراسات العيانية والمجهرية لساق وورقة نبات الرجـــــلة
والدراسة الكيميائية لمكونات النبـــات

هنـاء محمد سيـد ـ محمد عبد المطلب عبد الحافظ
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١ـ فى هذا البحث اجريت دراسة عيانية ومجهرية لساق وورقة نبات الرجـلة وهذه الدراسة تساعد على التعرف عليها.

٢ـ اثبت المسح الكيميائى للنبات وجود مواد كربوهيدراتية او جلوكوزيدات ستيرولات او تربينات ثلاثية ـ مواد فلافونيدية بالاضافة الى قلوانيـــات او مواد قاعدية ازوتيـــة.

٣ـ اسفرت دراسة المحتوى الدهنى باستعمال كروماتوجرافيا الطبقة الرقيقة وكروماتوجرافيا العمود وكروماتوجرافيا الغاز عما ياتى:

أـ وجود البيتا اميرين ـ بيتاسيتوستيرول ـ كامبستيرول وسنجماستيرول فى الجزء الغير قابل للتصبن.

بـ احماض دهنية مشبعة هى كابريك ـ لوريك ـ ميرستيك ـ ايزوبالميتيك بالميتيك وستياريك.

احماض دهنية غير مشبعة هى بالميتوليك ـ اوليك ـ لينوليك وحامضيـــن اخرين لم يمكن التعرف عليهما.

٤ـ تمت دراسة المواد القاعدية وامكن فصل مادة منهم وباستخدام كروماتوجرافيا الغاز المقرون بمطياف الكتلة تبين انها خليط من مركبين لم يمكن التعرف عليهما لقلة الكمية المفصولة من هذه المـــادة.

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